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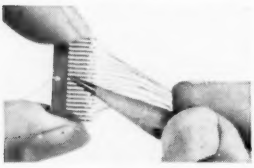

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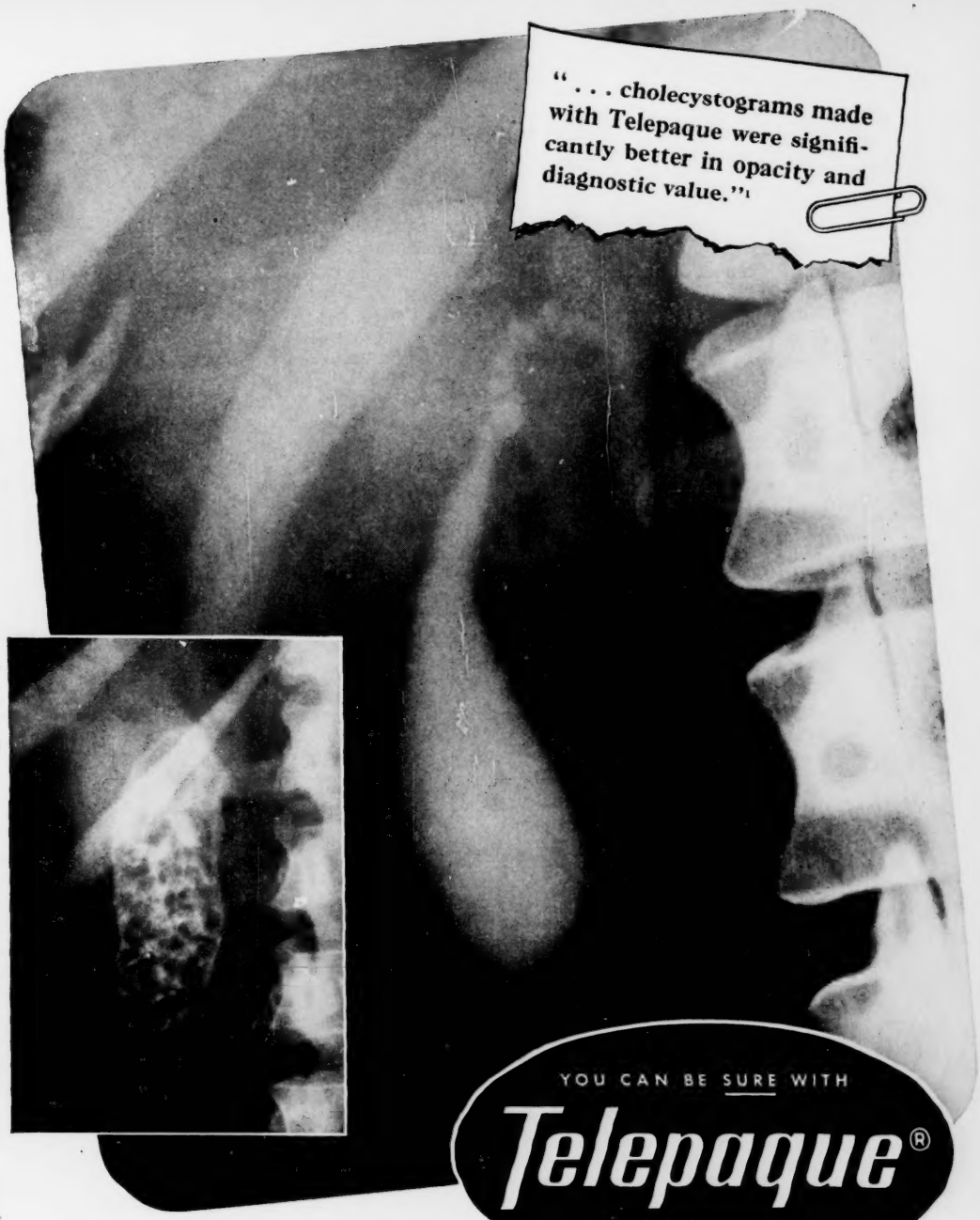
Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.



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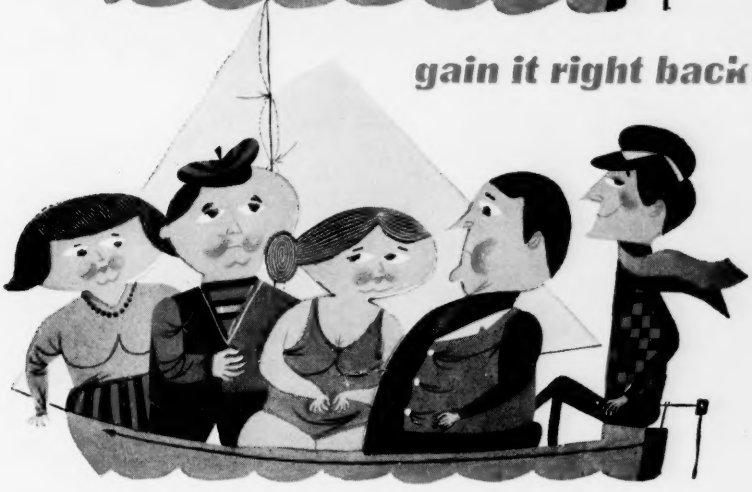

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DONALD MAINLAND | *Department of Medical Statistics, New York University College of Medicine*

THE RISE OF EXPERIMENTAL STATISTICS AND THE PROBLEMS OF A MEDICAL STATISTICIAN*

THE NARRATOR'S OBSERVATION POINT

If one desired a complete and objective history of a military campaign, one would hardly expect it to be written by a soldier on leave from his regiment which was still in action. His story could not be complete, and it would certainly overemphasize the activities of his own unit. And yet, are not the accounts of eye witnesses, of actual participants, the raw material from which histories are written? If, therefore, the title of this paper were fully descriptive, it would contain the phrase: "A contribution of raw material."

In gathering such raw material from an eye witness one should, of course, find out where he was when the events occurred, and so it is desirable to indicate briefly the experiences on which the remarks in this paper are based. The remarks are those of one who, after graduating in medicine, started research on the embryology of the ferret. He was puzzled by error in cell measurement, by variations in the counts of chromatin particles, and especially by the problems of small samples, because ferrets were expensive. He obtained no answer from biologists, chemists, physicists, or mathematicians, until he was led to the solution by Dr. C. H. Goulden, an agricultural experimenter in Winnipeg, who introduced him to the book by R. A. Fisher[4] (now Sir Ronald Fisher) on *Statistical methods for research workers* which had appeared three years previously (in 1925).

He then saw that the methods prescribed by Fisher for avoiding bias and allowing for chance, experimental error, biological variation, and sample size, were applicable in all fields of medicine, and that in his own field, anatomy, normal variations in nerve pattern, positions of abdominal viscera, and other organs had often greater clinical importance than the so-called "averages" that he had learned and was teaching. He stole time from the contemplation of the fifteen arteries, which (in those days, at least) arose from the hypogastric artery, and spent this time in applying statistics to his own research and the researches of others, some of it during vacations in Fisher's laboratory. Then, after World War II he realized that he could

* Presented at the annual meeting of the American Association of the History of Medicine, May 6, 1954, New Haven, Connecticut.

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not run an anatomy department and keep abreast of statistical developments, and he chose the latter effort.

This personal history will perhaps indicate where bias may occur in the remarks that follow, but the bias will, it is hoped, be more apparent than real. No one in medical statistics can ignore the debt to Raymond Pearl and his predecessors, who promoted a critical attitude toward medical data long before the rise of experimental statistics.

The brief autobiography serves also to illustrate what has happened during the past three decades to a number of workers in various fields of science, and here a kind of bias does enter. It can best be described in the words of a representative of a pharmaceutical house who came recently to the Department of Medical Statistics at New York University to discuss his search for a biological statistician. He said: "In my quest I have met many statisticians, and I have been struck by the enthusiasm of those who came into the field from the outside. I do not find the same enthusiasm among the younger people who have been formally trained as statisticians." Another way of expressing this would be to say that the older crowd, whatever their present professional status may be, are still "amateurs" in the original Latin sense of the word. The remarks that follow, therefore, will contain the bias of an amateur.

"STUDENT" AND FISHER

It was the patron saint of the amateur statisticians who wrote the paper that can be looked upon as the starting-point of what is called "experimental statistics," or, more exactly, "experimenters' statistics"—the paper that appeared in 1908 entitled "The probable error of a mean," by "Student,"[13] the pseudonym of W. S. Gosset, an experimenter employed at Guinness's Brewery in Dublin. In brewing experimentation "the variable materials and susceptibility to temperature change and necessarily short series of experiments" (McMullen[9]) showed, as Fisher[4] later expressed it regarding all laboratory investigation, that "the traditional machinery of statistical processes was wholly unsuited to the needs of practical research. Not only did it take a cannon to shoot a sparrow, but it missed the sparrow!"*

The method demonstrated by "Student" was later called the "t" test, and this led to the analysis of variance method for the comparison of two or more means, which was named the "F" test by Snedecor[12] in honor of Fisher who developed it.

* It is significant that in this quotation the present tense of the original can be changed to the past tense.

The next landmark in the presentation of statistics for experimenters was Fisher's *Statistical methods,*[4] already mentioned, which was produced while its author was at the Rothamsted Agricultural Experimental Station, and is now in its eleventh edition. Ten years later (in 1935) appeared his *Design of experiments,*[5] now in its sixth edition.

The far-reaching effects of these two books, and of Fisher's numerous papers, justify calling the past thirty years of statistics the Fisherian period (or sometimes the "Piscatorial" period); and the logical connection between the books is noteworthy. The first was concerned largely, though by no means wholly, with significance testing, i.e., with proper methods of making allowance for the effects of chance. The second book emphasized the fact that a significance test has no useful meaning unless an experiment has been properly designed. In terms of a simple experiment, the comparison of two treatments on animals or patients, "properly designed" means designed in such a way that, at the end of the experiment, one can say: "Chance would so rarely cause such a large difference in outcome that I shall attribute the observed difference to the treatments." There must be only two possibilities: chance and the treatments; and this situation can be reached only by allocating the treatments to the subjects by what Fisher[5] called "a physical experimental process of randomization," which is now most easily performed by a table of random numbers.

This demonstration of the logical necessity of randomization is one important contribution of Fisher's second book on statistics. Another is his demonstration of how to study multiple factors in the same experiment. He wrote: "In expositions of the scientific use of experimentation it is frequent to find an excessive stress laid on the importance of varying the essential conditions *only one at a time* . . . and it is often supposed that [this] is the essentially scientific approach to an experimental investigation. This ideal doctrine seems to be more nearly related to expositions of elementary physical theory than to laboratory practice in any branch of research." He further called attention to the way in which different factors interact with each other, and demonstrated designs that could most economically reveal not only the main effects but the interactions. In brief, the principles are: (i) balance, i.e., the subjection of equal numbers of subjects to each factor under test, and (ii) randomization of all the factors, known and unknown, that are not under test. (The economy possible by such methods can be illustrated by an experiment recently concluded on seven x-ray technique factors (kilovoltage, developing, fixing, and so on) in which only 128 films were needed, in contrast to 8,192 films that would have been required if the

so-called "scientific" method of varying only one factor at a time had been used.)

These and many other methods that Fisher developed are much more than experimental techniques. They were the first results of a revolution that is still continuing in the hands of others, a revolution by which statistics came to embody the principles of inductive inference—the experimenter's logic.

STATISTICS IN APPLIED SCIENCES

It is important to note where the statistical revolution started and where it spread—in applied sciences. It began in brewing and agriculture, spread to animal husbandry (as in the work of Snedecor[19] in Iowa), to the cotton industry in England, and now, either by research or statistical quality control or both, statistics plays a part at some stage in the production of probably everything that we use or consume. Noteworthy, because of its connection with medicine, is the pharmaceutical industry, where, especially in bioassay, some of the most valuable advances in statistical method have occurred.

In all the applied sciences, inefficient or wrong methods of research or production cause loss of money. Therefore, sound experimentation was profitable; and so applied chemistry and physics adopted modern biological statistics while academic chemists, physicists, and even biologists were disregarding the revolution or resisting it, largely through ignorance.

What, then, about medicine, which in a scale of human values may be considered the highest of the applied sciences? This is the field where quality of work is to be measured by something greater than loss or gain of dollars. It is the field, also, where there is much lamentation over the walls that have arisen between the various "subjects" in teaching and research and over the lack of basic principles of thought, such as are offered by modern statistics.

EXPERIMENTAL STATISTICS IN MEDICINE

The first example in "Student's"[18] paper of 1908 dealt with the data from a clinical trial of soporifics, but it could hardly have had less effect on medicine if it had dealt with a constellation in outer space. In 1929 there appeared in *Physiological Reviews* a 124-page article by H. L. Dunn[8] who reported that out of 200 quantitative medico-physiological papers in current American periodicals over 90 per cent required statistical methods and did not use them. The rest of the article was occupied with a description of statistical tests, mostly pre-Fisherian, with formulae and examples. This

article was distributed widely and doubtless helped to increase the incidence of statistical tests in medical literature. In 1932 Greenwood,[7] the English medical statistician, remarked that "medical papers now frequently contain statistical analyses, and sometimes these analyses are correct, but the writers violate quite as often as before, the fundamental principles of statistical or of general logical reasoning."

In the past twenty years the increase in the incidence of tests—statistical arithmetic—has continued, and so also, very commonly, has the disregard of the more important contribution of statistics, the principles and methods of sound, economical experimentation and valid inference. Of the various causes of this neglect, one has been clearly demonstrable in some investigators—the fact that medical science had its roots largely in academic physics and chemistry. Another obvious cause is the common human tendency to use gadgets instead of thought. Here the gadgets are the arithmetical techniques, and the statistical "cookbooks" that have presented these techniques most lucidly, without primary emphasis on experimentation and logic, have undoubtedly done much harm.

However, all has not been darkness. Lights have appeared in various laboratories and clinics. Thus, in the 1930's proper attention was given by Gaddum[6] in England and Bliss[2] in this country to the fact that individual animals of the same species, sex, age, and weight differ in their response to drugs and toxins. In the clinical field there appeared in 1948 a beacon or lighthouse beam—the report of the British Medical Research Council's[11] co-operative trial of streptomycin in pulmonary tuberculosis.

Experimentation in medicine is, therefore, improving, not only by devices such as randomization and the balancing of samples, borrowed from other biological fields, but by methods specially appropriate to investigation on human beings, such as the "double blind" method, to avoid bias both from the patient and the observer.

Much research on human beings must, however, remain nonexperimental —attempts to show the causes of disease, interrelationships between diseases, biochemical and other differences between them. Problems of biased samples in such research have long been the concern of public health statisticians, but the light of experimental statistics has shown the difficulties more vividly than before. One example has been called "Berkson's fallacy," to indicate the one who first demonstrated it, Dr. Joseph Berkson of the Mayo Clinic.[1, 9] The fallacy is not difficult to grasp, and a research worker in any field, from anatomy to psychiatry, who appreciates its implications, will be perturbed by it, for it may affect all his researches. This is not the place to describe it, or to discuss in detail other recent developments in medical

statistics, but for some discussion of such topics, reference may be made to a section of the latest volume of *Methods in medical research*.[10] In that section an attempt has been made to meet some current problems, especially the problem of a scarce commodity—experienced medical statisticians.

THE STATISTICIAN'S PROBLEMS IN MEDICINE

"Meeting problems." The phrase recalls a remark made by Dean Currier McEwen in 1950 during the discussion of plans for a Division of Medical Statistics at the New York University College of Medicine. Dr. McEwen's remark was: "There will be lots of problems; but it will be fun meeting them." That remark, reinforced by the helpful attitude of colleagues in other departments, has provided the motto for the first four years of the experiment at New York University.

The Division became a separate Department in 1953, and some have said that it is the first such department in a medical school on this continent, independent of a department or school of hygiene, public health, or preventive medicine. Whether that is so or not, the Department is probably a forerunner of other such units and is meeting problems that they will meet. Problems are inevitable when anything is established that does not fit an existing pattern, and such a department does not. It is an academic department, but resembles also a service unit, concerned with maintenance or supplies. The chief problems can be grouped under three heads: Lack of time, lack of money, and lack of personnel. All the problems entail misconceptions of the nature and functions of statistics, misconceptions that are understandable by reference to the foregoing remarks on the history of experimental statistics. In feeling one's way toward solutions of the problems, one must keep constantly in mind the true goal: To help to improve medical research.

Lack of time. In any active research center a medical statistician will, sooner or later, find himself submerged by requests for help. He will find that many investigators have no idea how long it takes to provide proper diagnosis and treatment for a case—a research project—even for one that looks quite simple; and they are naturally disappointed and perplexed when, after days or weeks of inquiry and thought, the statistician has to say: "No treatment is warranted."

It may be asked: "Would it not be more sensible to give a little help to a lot of projects than a lot of help to a few?" This is an extremely dangerous suggestion. The "little help," on superficial acquaintance with the project, is commonly worse than none at all. A significance test, which "rules out chance," makes the investigator feel that he has a clean bill of health, that

his results are "statistically proved." But chance is often the least important cause of wrong conclusions; and the "t's" and "sigmas" and "chi-squares" in medical literature are often just spurious science.

A statistician who refuses to do arithmetic unless he knows that it is justified, who tries, in a phrase reminiscent of the Hippocratic Oath, "to keep his art inviolate," will attempt to do as much work as is necessary on as many projects as possible. He will become harassed and will develop a feeling of guilt because he is unable to keep his promises and holds up other investigators' work, even for months.

Toward his assistants, also, he will feel guilt, which he may express somewhat as follows: "I am too old to care much about my own future, but I do care about the fate of my young assistant who needs to get publications out, to earn a higher degree, to obtain salary increases. His time is taken up by work for others. He plans an experiment, is constantly on call during the months when it is running, then analyzes the results and presents a report; and for all this he gets a half-line of acknowledgment. Even if his name were in the by-line whenever he had helped in the investigation, he should, as a member of an academic staff, have adequate time to work on his own problems. Should I recommend that he join a commerical company? Life there would not be free of pain, but a few thousand dollars' extra salary would provide a good analgesic. What should I do?"

The answer that seems to be emerging from experience is twofold:

1. Find out who, in any other academic department, spends the greatest number of hours in teaching. Subtract the teaching time of the statistician of comparable rank, and let the difference be the time that the statistician devotes to his or her "service" functions.

2. Schedule the "service" work at least six months in advance. Select the projects that you can handle adequately in the available time and that seem most likely to bring you nearest to your main goal, the improvement of medical research. Decline the rest. This will be unpleasant, for you will have to refuse help (apparent help) to your friends in projects that are valuable; but even if it were right to give the whole time of your department to this kind of work you would be far from meeting the need.

Lack of money. When a person starts a statistical unit in a medical school, he is likely to be given a very small staff and a small budget and to be told that, as in other departments, he will have to look elsewhere for further help. He will probably not find that help easily. When he explains what he is trying to do—develop the science and art of statistics, and train workers properly—he may meet a remark like that made in 1951 by a high official in one of the foundations for the aid of medical education and

research: "A statistician cannot show an experimenter how to do an experiment." What, then, should a statistician do to raise money? Should he charge his colleagues in other departments for his advisory services? No; even a charge for a computing clerk's services, to help pay her salary, can at times injure the relationship between colleagues.

One recommendation, again based on experience, would be: "If you have been an experimenter in any field, carry on experimental work, applying for grants as do other departments." (Before accepting an appointment it is well to stipulate that facilities be available for research of the kind contemplated.) Even if there were no need to raise money, the same recommendation would be made, for thereby is created the environment which, as a glance at recent history has shown, is best for the development of the science and art of statistics.

Lack of personnel. There is no simple or speedy solution for the problem of the scarcity of medical statisticians; but in seeking for a solution three points should be borne in mind:

1. The first point is essentially a repetition of the last remark in the preceding section. The most important single element in the training (and continuous education) of any statistician is practical experience—experience of investigations for which he himself is responsible, with all their difficulties and disappointments. It is, therefore, very satisfactory to note that in the new scheme of training at Yale—a most important experiment—the emphasis is on actual investigation and not on mathematics.

2. If medical schools hope to increase the supply of statisticians, they must see clearly what is implied. They should no longer imagine that any one person can be competent in vital statistics, experimental statistics, epidemiology, cost accounting, mathematical theory, and the analysis of hospital records (if they are worth analyzing).

3. Although, as time goes on, the supply of suitable statisticians will increase, they will not be the whole answer to the need for statistics in medicine, because the need is for better statistical reasoning by investigators. A course in statistics for medical students can go only a very short way toward meeting this need. It could go somewhat farther if students, in college and medical school, were shown the difference between a proper experiment and what is called an experiment in their laboratory courses, or if some of the elementary exercises in chemistry and physics were transformed into real experiments, or, perhaps, if children in kindergarten or elementary school became really familiar with chance and bias through games with marbles or disks.

A rather more hopeful method of meeting the need for statistical reasoning in medical research, which has apparently not been systematically tried, would be the development of a "statistical nucleus" in each department (or group of cognate departments in a small school). This nucleus would be one of the regular staff members (biochemist, physiologist, physician, or pathologist, according to the department) who would have a sound grasp of the general principles and of a few suitably chosen investigational designs, along with the methods of analyzing the resulting data. The designs would have to be simple, but that would, in itself, be a blessing, because many experiments in medicine are too complicated, too "ragged," and very wasteful. The success of the scheme would depend largely on two things:

1. The attitude of the head of the department and other investigators.

2. The characteristics of the person who was to be the "nucleus." He should really desire to learn and understand and should be able to rid his mind of mistaken ideas and prejudices. Although probably young, he should be mature enough to be patient with the notions of his seniors, but at the same time firm when he knew he was right. If in doubt, before committing himself he should call in a professional statistician.

DISCOURAGEMENTS AND REWARDS

The scheme just outlined would bring difficulties and discouragements, and indeed much of this paper may seem discouraging to one who would like to become a medical statistician. Therefore at the end let the voice of an amateur be heard again. The problems are numerous and heavy; but they seem trivial in face of the satisfaction—the thrill—that can come when several people are planning together a piece of research, when the distinctions between physician and surgeon, chemist and statistician, fade away, because all are trying to practise the difficult art of straight thinking—all are striving to create a good investigation.

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ACTION OF CATHEPSIN C ON DIPEPTIDE ESTERS*

In earlier studies[12] on the purification and properties of beef spleen cathepsin C, it was found that at pH 5 this proteolytic enzyme catalyzes the hydrolysis, not only of the CO-NH bonds of sensitive substrates, but also of the ester linkage of α-L-glutamyl-L-tyrosine ethyl ester. In view of the subsequent finding[4] that cathepsin C exhibits pronounced specificity toward dipeptide derivatives (*e.g.*, glycyl-L-phenylalaninamide [GPA]), but does not act on related amino acid amides (*e.g.*, L-phenylalaninamide) or tripeptide amides (*e.g.*, glycylglycyl-L-phenylalaninamide), it seemed of interest to examine more closely the action of the enzyme upon several dipeptide esters both at pH 5 and at pH 7.5. Previous work[9] had shown that at pH 5 the predominant reaction is one of hydrolysis at the sensitive amide bond of substrates such as GPA; at pH values near 7.5, the major reaction is one of transamidation[8] leading to the synthesis of long-chain polypeptides. Thus, at pH 7.5, GPA is converted by the enzyme to a polymeric product that is, on the average, an octapeptide composed of alternating glycyl and L-phenylalanyl residues.

In Table 1 are given the results of experiments in which several dipeptide esters were subjected to the action of two preparations of cathepsin C, kindly provided by Dr. Peter S. Cammarata and Dr. Gabriel L. de la Haba of this laboratory.† These two enzyme preparations differed in their specific activity toward GPA; preparation I contained 32.8 cathepsin units per mg. N, whereas preparation II contained 9.6 cathepsin units per mg. N.‡ It will be seen from Table 1 that when equal amounts of enzyme, as measured by the action on GPA, are used, the two preparations are equally

* This study was aided by grants from the Rockefeller Foundation, from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council), and from Eli Lilly and Company.

† Recently, an improved method for the purification of beef spleen cathepsin C has been developed in this laboratory. The procedure involves autolysis of the tissue at pH 3.5 and 38°, followed by fractional precipitation with ammonium sulfate and with acetone. The details of the purification method will be presented in a separate communication.

‡ The definition of this unit, and the method for the determination of cathepsin activity toward GPA, are described in an earlier paper.[12]

Received for publication May 10, 1954.

effective on the ester analog of GPA, glycyl-L-phenylalanine ethyl ester (GPE). The fact that GPE or glycyl-L-tyrosine ester (GTE) is hydrolyzed at approximately the same rate at pH 5 by the two enzyme preparations may be taken as evidence for the view that these dipeptide esters are attacked by the same enzyme that hydrolyzes GPA at this pH. This result suggests

TABLE 1. ACTION OF CATHEPSIN C ON ESTERS OF PEPTIDES AND OF AMINO ACIDS

Substrate concentration, 0.05 M; concentration of cathepsin C, 0.4 units* per ml.; 0.1 M citrate (pH 5.1) or phosphate (pH 7.6) buffer; cysteine concentration, 0.004 M; total volume, 2 ml.; temperature, 38°.

		Disappearance of ester†				
		pH 5.1		*pH 7.6*		
Substrate	*Time*	*Prep. I*	*Prep. II*	*Prep. I*	*Prep. II*	*No enz.*
	min.	*%*	*%*	*%*	*%*	*%*
Glycyl-L-phenylalanine ethyl ester	40	13	18	51§	55§	22
	60	23	28	56§	60§	25
	120	35	40	65§	66§	38
Glycyl-L-tyrosine ethyl ester	60	13	18	63	60	27
	120	28	37	73§	71§	42
Glycylglycine ethyl ester	20	19	15	42	45	27
	60	48	50	62	62	48
	120	59	68	66	65	61
β-Alanyl-L-phenylalanine ethyl ester	120	1	1	0	0	0
β-Alanyl-L-tyrosine ethyl ester	120	0	0	3	3	0
Glycine ethyl ester	240	0	0	14		9
L-Phenylalanine ethyl ester	240	2		10		7
L-Tyrosine ethyl ester	240	1		10		7

* Two enzyme preparations of different specific activity were employed. Preparation I had a specific activity of 32.8 cathepsin units per mg. N, and preparation II had a specific activity of 9.6 cathepsin units per mg. N, as determined by the rate of deamidation of glycyl-L-phenylalaninamide.[19]

† Determined by a modification of the hydroxamic acid method of Hestrin (for details, see experimental section).

§ A precipitate was present in the incubation mixture.

that cathepsin C, like several other proteinases (*e.g.*, trypsin, chymotrypsin[11]), can act as an esterase at suitable CO-OR bonds, in addition to its behavior as a peptidase that cleaves CO-NH linkages.

It is worthy of note that the cathepsin C preparation I did not cause appreciable hydrolysis of L-phenylalanine ethyl ester or of L-tyrosine ethyl ester under the same experimental conditions as those used for the dipeptide esters. This indicates that the action on the dipeptide esters cannot be attributed to a non-specific esterase. In addition, replacement of the glycyl residue in GPE or in GTE by a β-alanyl residue, to give either β-alanyl-L-phenylalanine ethyl ester or β-alanyl-L-tyrosine ethyl ester, prevents the enzymic hydrolysis of the ester linkage. It would appear, therefore, that the action of cathepsin C at pH 5 is narrowly restricted to the hydrolysis of ester or amide bonds of the appropriate derivatives of dipeptides composed of two α-amino acid residues. This may be taken to imply that, for the catalytic action of cathepsin C on one of its substrates, the free α-amino group and the carbonyl group of the sensitive ester or amide linkage must be located in the substrate at a precisely defined distance from each other. To account for the stereochemical specificity of cathepsin C, which acts only on the L-isomer of GPA,[4] it is necessary to assume at least one more point of interaction between the substrate and the active group of the enzyme.[2]

The data in Table 1 also show that at pH 7.6 cathepsin C catalyzes the formation of an insoluble precipitate when GPE or GTE is used as a substrate. The chemical nature of these products has not yet been established, but, in view of the earlier findings with dipeptide amides, it is reasonable to assume that these insoluble precipitates represent long-chain peptides formed by the enzyme-catalyzed polymerization of the dipeptide esters. In the absence of cathepsin C, no such precipitates are observed under the conditions of these studies. It will be seen, however, that at pH 7.6, the dipeptide esters disappear from the solution even in the absence of enzyme. This may be attributed to the well-known tendency of such compounds to form diketopiperazines in alkaline solution. Further studies are required to establish the extent to which diketopiperazine formation is a factor in the enzymic experiments at pH 7.6. It may be added that at pH 5, in the absence of cathepsin C, no disappearance of ester was noted during the time period used in the enzymic studies.

The dipeptide esters containing a β-alanyl residue, and the amino acid esters, which were resistant to enzymic hydrolysis at pH 5, also were not significantly affected by cathepsin C at pH 7.6. The slow disappearance of the amino acid esters at pH 7.6 may be attributed to diketopiperazine formation.

It was a source of some surprise to find that glycylglycine ethyl ester (GGE) is an excellent substrate for cathepsin C at pH 5, and that some insoluble material was slowly formed at pH 7.6, although at the more alkaline pH value there was extensive diketopiperazine formation. The fact that both cathepsin C preparations attacked GGE at approximately the same rate at pH 5 suggests that this substance is hydrolyzed by the same enzyme that cleaves GPA and GPE and indicates that the side chain specificity of cathepsin C is not restricted to the aromatic side chains of phenylalanine and tyrosine.

In view of the result with GGE, a study was made of the action of cathepsin C on glycylglycinamide (GGA). It will be seen from Table 2 that GGA is deamidated by the enzyme at pH 5, although the rate of this reaction is extremely slow when compared with the action on GPA. When cathepsin C acts on GGA at pH 7.6, there is also observed a slow deamidation, but this is accompanied by the appearance of an insoluble precipitate, presumed to be a polymer formed by enzyme-catalyzed transamidation. This conclusion is supported by the fact that the extent of the reaction at 20 hours, as measured by the hydroxamic acid test, is greater than the extent of disappearance of amino-N. Further studies are required to establish more definitely the nature of the insoluble product, but it should be noted that its formation is not observed in the absence of cathepsin C and that there is negligible deamidation under these conditions.

It should be added that earlier experiments with crystalline chymotrypsin[8] had shown that this enzyme causes a slow cleavage of GGA, and it will be of interest, therefore, to examine the action of this enzyme on GGE.

Earlier studies had shown[12] that at pH 5 partially purified cathepsin C acts on L-phenylalanylglycinamide (PGA) or L-tyrosylglycinamide (TGA), but much more slowly than on GPA or on glycyl-L-tyrosinamide (GTA). These earlier data, when added to the results reported in the present communication, justify the conclusion that although cathepsin C is narrowly restricted in its action with respect to its "backbone specificity,"[2] the enzyme exhibits relative specificity with respect to the nature of the amino acids present in the dipeptide amide or ester. Since the dipeptide amides appear to be attacked less readily than are the corresponding dipeptide esters, as shown by a comparison on the rates of hydrolysis of GGE and GGA at pH 5, the relative "sidechain specificity" is more evident for a series of dipeptide amides than for a series of dipeptide esters. Thus, the fact that GPA or GTA is attacked more rapidly by cathepsin C than is GGA, PGA, or TGA indicates that the enzymic action is favored by the participation of an aromatic amino acid in the sensitive CO-NH linkage.

This relative specificity tends to be obscured when the cathepsin C acts on dipeptide esters such as GGE, GPE, and GTE, all of which are rapidly cleaved by the enzyme.

To the various dipeptide amides now known to undergo polymerization reactions in the presence of cathepsin C at pH values near 7.5 may be added L-seryl-L-phenylalaninamide (SPA) and L-seryl-L-tyrosinamide (STA). As will be seen from Table 2, these two compounds are deamidated at pH 5 without the appearance of insoluble products, whereas at pH 7.6 there is very rapid appearance of such precipitates. In view of the earlier results[4]

TABLE 2. ACTION OF CATHEPSIN C ON DIPEPTIDE AMIDES

Substrate concentration, 0.05 M; concentration of cathepsin C,* 0.4 units per ml.; 0.1 M citrate (pH 5.1) or phosphate (pH 7.6) buffer; cysteine concentration, 0.004 M; total volume, 2 ml.; temperature, 38°. Unless otherwise stated, the extent of the reaction was measured by means of ammonia determinations.

		Extent of reaction		
			pH 7.6	
Substrate	*Time*	*pH 5.1*	*with enz.*	*no enz.*
	hrs.	%	%	%
Glycylglycinamide	1	2†	2†	1†
	4	6†	6† (3§)	1†
	20	54†	49† (21§)	3† (4§)
L-Seryl-L-phenylalaninamide	1	15	22‡	0
	2	27	28	1
	4	38	29	1
L-Seryl-L-tyrosinamide	1	15	23‡	0
	2	25	25	0
	4	34	27	0
Carbobenzoxy-L-seryl-L-phenylalaninamide¶	4	0	0	
Carbobenzoxy-L-seryl-L-tyrosinamide¶	4	0	0	

* Specific activity, 32.8 cathepsin units per mg. N.

† As measured by the modified hydroxamic acid method (for details, see experimental section).

§ As measured by the Van Slyke nitrous acid method.[10]

‡ An insoluble precipitate had appeared in the incubation mixture by this time.

¶ This substrate was tested at 0.025 M in the presence of 30% ethanol.

on the enzyme-catalyzed polymerization of dipeptide amides, it is justifiable to conclude that these insoluble products represent polymeric peptides formed by transamidation. Studies are in progress to determine the chain length of these polymers and to effect substitution at the side chains of the component amino acid residues.

It will also be noted from Table 2 that the carbobenzoxy derivatives of SPA and of STA are not attacked by cathepsin C either at pH 5 or at pH 7.6. This result is in accord with the earlier conclusions concerning the specific requirement, by cathepsin C, for a free α-amino group in the dipeptide derivatives that it attacks.

DISCUSSION

The demonstration that animal tissues such as beef spleen contain an enzyme that catalyzes the polymerization of dipeptide units to form long-chain peptides raises the question whether this enzyme plays any physiological rôle in the elongation of peptide chains during the biosynthesis of proteins. Although no evidence is available at present for or against this possibility, it should be emphasized that cathepsin C is the first well-defined enzyme shown to catalyze the formation of long-chain peptides under physiological conditions of pH and of temperature. It appears likely that other intracellular proteinases of animal tissues will be found to cause similar polymerization reactions. The fact that not only dipeptide amides, but also dipeptide esters are substrates for cathepsin C suggests the possibility that compounds of the latter type may be intermediates in protein synthesis. It will be important to test these working hypotheses by the administration of suitably labeled dipeptide derivatives to biological systems in which protein is being synthesized.

For further progress in the understanding of the mode of action of cathepsin C, a highly purified preparation of the enzyme is needed. Work toward this end is in progress in this laboratory; the availability of a homogeneous enzyme preparation will permit a more precise study of the kinetics and the specificity of cathepsin C. It will be of special interest to examine the manner in which such an enzyme preparation attacks protein substrates, since it may be expected that at pH 5 the cleavage of proteins should lead to the formation of dipeptides in a manner resembling the action of β-amylase in releasing maltose units from an amylose chain.

The polymeric products obtained thus far by the action of cathepsin C on dipeptide derivatives are insoluble in water. Experiments are currently in progress to convert some of these products to soluble polypeptides by chemical treatment (*e.g.*, phosphorylation of the L-seryl-L-phenylalanyl polymer).

Such soluble polypeptides will be of value as possible model substrates for proteinases and for other enzymes; it will also be of interest to examine their behavior in various biological systems.

EXPERIMENTAL

Measurement of extent of reaction. The procedure used to follow the disappearance of ester is based on the hydroxamic acid method of Hestrin,[6] and was conducted as follows. Each of two 0.1 ml. samples of the incubation mixture was diluted with 2 ml. of a 1 : 1 mixture of 3.5 N NaOH and of 2 M hydroxylamine hydrochloride. After four minutes at room temperature, there were added, with shaking, 1 ml. of 4 N hydrochloric acid and 1 ml. of 0.74 M $FeCl_3$ in 0.1 N hydrochloric acid. The transmission of the red solution was read in a Bausch and Lomb photoelectric colorimeter, using a 550 mμ filter. A blank solution was prepared with 0.1 ml. of water and the above reagents. For each ester, a standard curve was prepared, relating light transmission to concentration of ester, thus permitting the calculation of per cent disappearance of ester in the enzyme experiments.

It was found that the above method could also be used to follow the disappearance of GGA during the action of cathepsin C. With this amide, however, it is necessary to incubate the 0.1 ml. sample with the alkaline hydroxylamine solution for a longer time period. In the determinations reported in Table 2, the hydroxamic acid formation was allowed to proceed for 25 minutes at 37°. In addition to the use of the hydroxamic acid method, the disappearance of GGA was also followed by means of the Van Slyke nitrous acid method, as modified by Kendrick and Hanke.[10]

The action of cathepsin C on SPA, STA, and their carbobenzoxy derivatives was followed by measurement of the extent of ammonia liberation in Conway microdiffusion vessels.[8]

Glycyl-L-phenylalanine ethyl ester hydrochloride. 2.0 gm. of the corresponding carbobenzoxy compound[7] were hydrogenated in ethanol in the presence of palladium black and 1.2 equivalents of concentrated hydrochloric acid. After hydrogenolysis, the mixture was filtered, and the filtrate was concentrated *in vacuo* to yield 1.4 gm. of crystals; $[\alpha]_D^{29} + 7.2°$ (2% in water).

$C_{13}H_{19}O_3N_2Cl$	Calculated	C 54.4,	H 6.7,	N 9.8
286.8	Found	C 54.2,	H 7.0,	N 9.6

Glycyl-L-tyrosine ethyl ester acetate. This compound was prepared from 2.0 gm. of the corresponding carbobenzoxy compound[1] in the same manner as above, except that glacial acetic acid was used in place of hydrochloric acid. Yield 1.5 gm.; $[\alpha]_D^{29} + 16.2$ (2% in water).

$C_{15}H_{22}O_5N_2$	Calculated	C 55.2,	H 6.8,	N 8.6
326.3	Found	C 55.0,	H 7.0,	N 8.3

β-Alanyl-L-phenylalanine ethyl ester hydrochloride. Carbobenzoxy-β-alanine (2.2 gm.) was dissolved in a mixture of 25 ml. of methylene chloride and 1.7 ml. of triethylamine. The mixture was cooled to —3° for 15 minutes, and then 1.1 gm. of ethylchlorocarbonate was added. After a further 30 minutes at —3°, there was added a chilled solution of 2.3 gm. of L-phenylalanine ethyl ester hydrochloride in a mixture of

25 ml. of methylene chloride, 10 ml. of chloroform, and 1.7 ml. of triethylamine. The reaction mixture was stirred continuously for four hours at room temperature and allowed to stand overnight. Water (50 ml.) was added, and the methylene chloride-chloroform layer was washed successively with dilute bicarbonate, dilute hydrochloric acid, and water. The organic solvent layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield 4 gm. of crystals, which melted at 69-70° after recrystallization from ethyl acetate-petroleum ether. Calculated for $C_{22}H_{26}O_5N_2$ (398.4), N 6.9; found, N 6.7. This carbobenzoxy compound (2.0 gm.) was hydrogenated in the usual manner in the presence of 1.2 equivalents of concentrated hydrochloric acid. Yield, 1.5 gm.; calculated for $C_{14}H_{21}O_3N_2Cl$ (300.8), N 9.3; found N 9.1.

β-Alanyl-L-tyrosine ethyl ester hydrochloride. This compound was prepared in the same manner as the phenylalanine analog. The yield of the carbobenzoxy compound was 4.0 gm.; m.p., 138-139°; calculated for $C_{22}H_{26}O_6N_2$ (414.4), N 6.8; found, N 7.0. Upon hydrogenation of 2 gm. of the carbobenzoxy compound, 1.2 gm. of the desired product was obtained. Calculated for $C_{14}H_{21}O_4N_2Cl$ (316.8), N 8.8; found, 8.5.

Glycylglycinamide acetate. This compound was prepared in the manner described previously.[3]

L-*Seryl*-L-*phenylalaninamide hydrochloride.* 1.75 gm. of carbobenzoxy-L-serinhydrazide was converted to the azide and coupled with L-phenylalanine ethyl ester (from 1.6 gm. of the hydrochloride) in the manner described previously.[5] The resulting syrupy ester was treated with ammonia in methanol to give 1.4 gm. of the carbobenzoxydipeptide amide; m.p., 189°; calculated for $C_{20}H_{23}O_5N_3$ (385.4), N 10.9; found, 10.9. Hydrogenolysis in the presence of 1.2 equivalents of hydrochloric acid gave the desired product; $[\alpha]_D^{29}$ + 26.7° (2% in water).

$C_{12}H_{18}O_2N_3Cl$	Calculated	C 50.1,	H 6.3,	N 14.6
287.7	Found	C 50.3,	H 6.5,	N 14.4

L-*Seryl*-L-*tyrosinamide hydrochloride.* This compound was prepared in the same manner as the phenylalanine analog. The carbobenzoxydipeptide amide melted at 200°; calculated for $C_{20}H_{23}O_6N_3$ (401.4), N 10.5; found, N 10.7. The dipeptide amide hydrochloride had a specific rotation of $[\alpha]_D^{29}$ + 30.0° (2% in water).

$C_{12}H_{18}O_4N_3Cl$	Calculated	C 47.5,	H 6.0,	N 13.8
303.7	Found	C 47.8,	H 6.0,	N 13.8

ACKNOWLEDGMENT

The authors wish to acknowledge with thanks the valuable assistance of Mr. Jack Graff.

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THE ORIGIN OF THE TERM *RIDGELING**

The biological term *ridgeling* is of uncertain derivation.[35] The word has been applied to various domestic animals; most commonly it refers to a male which has been "half castrated," i.e., has had only one testis removed, the other gonad being cryptorchid and relatively inaccessible to the operator. For reasons to be explained, such animals are useless and are often unmanageable and vicious.

Normally, the testis, which develops embryologically near the kidney, descends toward the end of fetal life or in the first year after birth into the pelvis, leaves the body cavity via the inguinal canal, and comes to rest in the scrotum. In cryptorchidism, a not uncommon condition,[27] the testis does not complete this journey, and may not even enter the inguinal canal. It appears from a search of the literature that removal of cryptorchid testes from animals was seldom attempted until relatively recently; lack of surgical skill and the hazard of infection deterred the farmer or stock breeder who could perform a simple castration.[7, 10, 34, 40]

The ridgeling horse or sheep or dog, having lost his normal testis but retaining an abnormally located gonad, develops atypically. He is sterile, for cryptorchid testes seldom if ever produce sperm; hence he is worthless for breeding. He develops the sex drive of the uncastrated adult male, since the cryptorchid testis retains at least some of its endocrine function. The ridgeling may become unmanageable or even vicious,[10, 25, 34, 39, 50] partly because cryptorchidism may be painful.[14]

Less frequently, the term *ridgeling* described a cryptorchid animal which had *not* been castrated.[1, 8, 25, 26, 27, 36, 38, 51] Such beasts were also of little or no value; if unilaterally cryptorchid, they were fertile but were not bred, as it was feared that the anomaly might be inherited;[50] animals retaining both testes in the abdomen or inguinal canal were sterile anyway. In both cases, the behavior would not be that of the docile castrate but of the normal, aggressive male, and the animal might be savage as well.[10, 25, 34, 50] Small wonder that the unfortunate ridgeling was very unpopular indeed.

* The author is indebted to *A New English Dictionary on Historical Principles*[37] and to Professor Hans Kurath, editor of the forthcoming *The Middle English Dictionary*, for mention of many of the original illustrative passages. Professor G. Lincoln Hendrickson kindly translated the lines from Theocritus, and Professor E. Talbot Donaldson gave assistance with several etymological questions.

The *Sporting Magazine* for 1811 reported an "Interesting Horse Cause," or lawsuit, brought to recover the price paid for a horse which, although it had been sold as a gelding, could not be pastured with mares. It developed that this animal was a ridgeling and that two attempts had been made to remove the cryptorchid gonad.[2] Uncontrollable behavior, especially in the presence of mares, is common in ridgeling horses; they may injure themselves or others, and are almost worthless.[10, 25, 34, 50]

Cryptorchidism is said to be more common in Angora goats (approximately 5.5%) than in any other domestic animal.[3, 34] Mention of a ridgeling goat occurs in an interesting instance of apparent plagiarism. Idyll III of Theocritus (3d century B.C.) includes a reference to a goat: *χαὶ τὸν ἐνόρχαν τὸν Λιβυχὸν χνάχωνα Φυλὰσσεο, μὴ τὺ χορυΨῃ.* [and beware of the yellow Libyan he goat lest he butt thee with his horns.][58] As translated by Zamagna in 1792 into Latin, the lines become *Namque caper Libycus cornu ferit ille, caveto* [Beware the Libyan goat, for he bears horns.][58] Now a passage in the eleventh Eclogue of Virgil (70-19 B.C.) is similar to Theocritus' original; Virgil's *occursare capro* (*cornu ferit ille*) *caveto*[45] would seem to have influenced Zamagna's much later translation.[58] To make matters more complicated, Thomas Creech (1659-1700), in translating this passage from Theocritus,[18] and John Dryden (1631-1700), in translating the corresponding passage from Virgil,[16] wrote almost identical lines ["But 'ware the Libyan Ridgling's butting Head." "And 'ware the Libyan ridgil's butting head."] which were also very free translations. One is glad to report that later translators were more accurate.[22, 43]

Canine ridgelings were recorded at least as early as 1702;[31] the condition is not uncommon in dogs[14, 32] or boars.[50] The ridgeling boar, called in a German dialect *Rig* or *Rigel,*[24, 38] as an adult has meat too strong for human consumption,[50] cannot be bred, and hence is worthless. An incompletely caponized fowl was known in Austria as a *Rigler* or *Halbhahn;*[23, 24, 38] the similarity of the former term to *Rig* and *Rigel* undoubtedly related to the fact that in the half-caponized fowl as in the ridgeling boar one testis is left in the abdomen, although for different reasons. Variants of *ridgeling* were even applied loosely to men—e.g., "redgelinges, or guelte [gelded] men";[9] "I hate a base cowardly Drone, / Worse than a Rigil with one Stone."[12] At least two current medical dictionaries[15, 47] support the application of the term to a man with one testis. John Hunter referred to a cryptorchid bull as a *ridgill.*[26]

Ridgeling sheep were troublemakers in any flock. One British community in 1597 had a law barring such animals from the common grazing ground:

Item a payne [penalty] sett that no personne or personnes shall put any *ridgell tupp* [ridgeling sheep] upon the moore between the feast of St. Luke the Evangelist and the natyvitie of *our* Lord god upon payne for every ridgell so found (Holmesfield Court Rolls).[1]

One hundred and fifty-four years later the language of the law had changed, but not its intent:

A pain set that any person or persons that shall keep any ridgell or close tupp [bilaterally cryptorchid ram] upon the moor or common from the first day of September untill the last day of December each person for each offence three shillings and four pence.[1]

During the 1640's one Henry Best, a Yorkshire farmer, kept careful records of his farming activities. Best, who clearly was an educated man, begins his very interesting journal with a discussion of sheep. He defines normal males, and then moves on to cryptorchidism.

Close tuppes are such as have both the stones in the ridge of the backe, and are therefor very difficult to geld. Riggon tuppes are such as have one stone in the codde, and the other in the ridge of the back, and therefore the most dainger and difficultie is in geldinge of these, beinge to be cutt in two places before they can be made clean weathers.[7]

Classical descriptions of the ridgeling condition, particularly as it occurred in sheep and cattle, form the basis for one supposed derivation of *ridgeling*. Marcus Terentius Varro (116-27? B.C.), in his *De re rustica*, advised the herdsman that periodically the herd must be examined and counted and that it should be decided how many worthless animals should be removed [*quot reiculae sint alienandae*].[48] Cattle which are useless should be eliminated [*reiculae reiciundae*] to make a place for animals of value.[33, 48] Nonius Marcellus, the classical grammarian, lists *reicula*, but applies it to "sheep culled out, whether for age or serious sickness" [*Reiculas oves, aut aetate aut morbo graves*].[30] Samuel Johnson[30] and numerous other eighteenth and nineteenth century lexicographers[5, 6, 18, 30, 30, 42] believed that *ridgeling* is derived from *rejicula* or *reicula*. There is something to be said for this opinion; the first syllables are similarly pronounced, and both end in a diminutive (*-ling, -ulus*). The author has found no evidence, however, that *reicula* referred specifically to a ridgeling, although the herdsman would certainly wish to remove ridgeling sheep or cattle from his flock. Another reason for doubting that ridgeling derived from the Latin *reicula* is that the latter term appears to have no direct descendant among the Romance languages.

Webster's Dictionary,[38] the "Oxford Dictionary,"[37] and *The English Dialect Dictionary*[51] agree that *ridgeling* and at least some of its variants* are apparently derived from *ridge,* an old term for the spine or back, close to which the cryptorchid testis was thought to remain. Certainly in the sheep and goat the cryptorchid testis is said to lie near the spine and the kidneys (which is where the fetal gonad normally develops prior to its migration into the pelvis). Henry Best in 1641 (see above) spoke of "Riggon tuppes," male sheep which "have one stone in the codde, and the other in the ridge of the back."[7] Lush, Jones, and Dameron, in a recent study,[34] state that "nearly always" in goats and sheep the cryptorchid testis "remains in its original position high in the abdominal cavity just posterior to the kidneys and slightly ventral to them. . . ." (page 5). Thomas Blount, an early lexicographer, in his *Glossographia: or, a Dictionary Interpreting the Hard Words,* published in 1674, says that a "Ridgil is the male of any beast" which is half castrated; "others add that also to be a *Ridgil,* whose stones never came down; but lie in his reins."[9]

On the other hand, it is reported that cryptorchid testes in the dog are usually found in or near the inguinal canal.[14, 32] In the horse, according to modern works on veterinary surgery, the cryptorchid testis most frequently lies on the *ventral* abdominal wall, near the pelvic brim.[10, 19, 40] Hobday has reported that in 77 horses on which he operated for cryptorchidism a retained testis was in the abdomen in 39 cases and in the inguinal canal in 43 cases.[25] (Since no reference has been found in the early literature to cryptorchid testes in the inguinal canal it is probable that this site was not a factor in the etymology of *ridgeling.*)

In summary, then, the idea that *ridgeling* derived from *rejicula* or *reicula* is tempting but is not supported by modern etymologists. The derivation from the *ridge* of the back of *ridgeling, ridgel,* etc. appears correct if the latter terms were first applied to sheep and goats, but seems less appropriate in the case of dogs and horses. More evidence would be helpful.

In some parts of the United States a *ridge runner* is a wild horse which may be seen running on the ridge of a hill.[49] In West Virginia, Ohio, and Missouri a *ridge runner* is an undisciplined adolescent or a semicastrated, cryptorchid farm animal. Asked why, an old farmer explained, "Because you can't keep it down in the valley with the herd; it's always running up on the ridge."[21]

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*Variants of ridgeling include: redgelinge, reggil, ridgel, ridgelin, ridgell, ridger, ridgil, ridgill, ridgillon, ridgiour, ridgit, ridgul, rig, rigel, rigeld, rigele, rigell, rigelle, riggald, riggelt, riggil, riggilt, rigg'lt, riggold, riggon, riggot, rigil, riglan, rigland, riglin, rigling, rigsie, rigwiddie, rogel, rudgel, rudger.[1–4, 6, 7, 11, 13, 16, 17, 25, 26, 28, 29, 35, 37, 38, 41, 44, 46, 51, 52]

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THE TRANSPORT OF OXYGEN IN THE SHEEP'S PLACENTA: THE DIFFUSION CONSTANT OF THE PLACENTA*

The problem of how oxygen is transferred from lung alveoli to blood was considered in 1909 by Christian Bohr,[8] who used the calculus to develop the appropriate equation for the diffusion of oxygen from alveoli into blood and presented a graphical solution. In this way, the integrated mean oxygen pressure gradient causing oxygen transfer across the alveolar membrane† was obtainable and, in addition, the diffusion constant of the lungs. While the theoretical basis was then at hand, refinements in experimental techniques were needed to yield further knowledge of the transfer of oxygen in the lungs. Lilienthal, Riley, *et al.*[6] have contributed substantially to the understanding of this process and their paper refers to other notable contributions since Bohr's.

The pulmonary methods of Bohr do not seem to have been applied to the more complex transport of oxygen from maternal to fetal blood, although the data for such an application, in the case of the sheep, are now at hand.[1,2]‡ We shall develop the theory of diffusion across the placental membrane separating maternal and fetal blood in the sheep and then apply it to a sample set of the data available.

ANALYSIS OF DIFFUSION OF OXYGEN IN SHEEP'S PLACENTA

Inherent in the development of a theory is the choice of simplifying abstractions to condense the actual complex situation into an analogous one more easily handled. Our basic assumption for the purpose of studying gas transport in the placenta of sheep is that the complex network of two sets of contiguous vessels can be adequately represented by two single vessels, each of uniform (though not the same) bore, equal in length, and separated by intervening tissue such that the rate of gas diffusion from any point of one vessel to the other is proportional to the pressure gradient of the gas

* An abstract of this paper has been published elsewhere.[5]

† The integrated mean oxygen pressure gradient across the alveolar membrane is that oxygen pressure difference which, if maintained along the entire length of alveolar capillaries, would cause the same rate of oxygen transfer as actually diffuses under biological conditions.

‡ Barron and Alexander analyzed their data by an inexact graphical method.

Received for publication May 12, 1954.

dissolved in the blood within the two vessels at the point of contiguity, the constant of proportionality being everywhere the same. Furthermore, the effects on the hemaglobin dissociation curves of the shift in pH resulting from the placental respiratory changes will be neglected since here they are not of quantitative importance and an exact treatment is difficult to handle. The data relied on were determined for a CO_2 tension of 40 ± 2 mm. Hg.

In Figure 1 is a simplified representation of the vessels of the placenta, the maternal and fetal directions of flow being opposite.

Small letters refer to the fetal circulation; capital letters refer to the same thing in the maternal circulation and are not set forth separately.

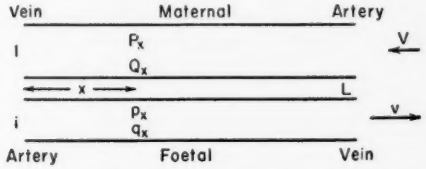


FIG. 1. Simplified version of the sheep's placental circulation used in the analysis of oxygen diffusion from maternal to fetal blood. Uniform bore vessels for both circulations, separated by tissue of constant resistance to oxygen diffusion, are conjectured. The arrows labelled V and v show the average linear velocity of flow as well as its actual direction. The dimensions are not supposed to be relatively correct; they are arbitrary. The symbols are defined in the text.

Let i = blood flow rate through fetal capillaries in cc./min.
v = length of capillary traversed by a sample of fetal blood per minute. (Linear velocity.)
a = cross-section in $cm.^2$ of fetal placental capillaries.
x = distance in cm. along capillaries, starting at arterial end of fetal capillaries, in the ideallized system of Fig. 1.
L = total length of placental capillaries, the maximum value of x.
p_x = O_2 partial pressure in mm. Hg of fetal blood when it has reached the point x.
q_x = vol. % of O_2 in fetal blood at x.
k = oxygen diffusion constant of placenta, i.e., cc. of O_2 transported from maternal to fetal blood per mm. Hg O_2 pressure difference per minute.
m = oxygen consumption of fetus in cc. per minute.

Consider fetal blood, as it passes from x to x + Δx, an adjacent point along the capillary pathway. (See Fig. 1.) Its increase in O_2 content in vol. % is Δq. Applying the definition of k and in the light of our premises:

$$k = \frac{\overset{(1)}{\Delta q \times \text{vol. of fetal blood in } \Delta x \text{ in 100 cc. units}}}{\underset{(2)}{\text{frac. of placenta rep. by } \Delta x} \times \underset{(3)}{\text{pressure diff.}} \times \underset{(4)}{\text{time for blood to go } \Delta x.}}$$

(1) $= a\Delta x/100$; (2) $= \Delta x/L$; (3) $= P_x - p_x$; (4) $= \Delta x/v$.

Since $i = av$,

$$\Delta q = (P_x - p_x)\frac{100k}{iL}\Delta x,$$

and, when $\Delta x \to o$, $\frac{dq}{dx} = \frac{100k}{iL}(P_x - p_x)$.

Correspondingly, for the maternal circulation, which is opposite in direction to that of the fetal circulation,

$$\frac{dQ}{dx} = \frac{100k}{IL}(P_x - p_x)$$

When these two equations are combined to eliminate dx and then integrated to obtain the relationship of q_x to Q_x, one obtains:

$$Q_x - Q_o = (q_x - q_o)\ \frac{Q_L - Q_o}{q_L - q_o},$$

or

$$Q_x = Q_o + (q_x - q_o)\ i/I.$$

Returning to the expression for $\frac{dq}{dx}$ and integrating,

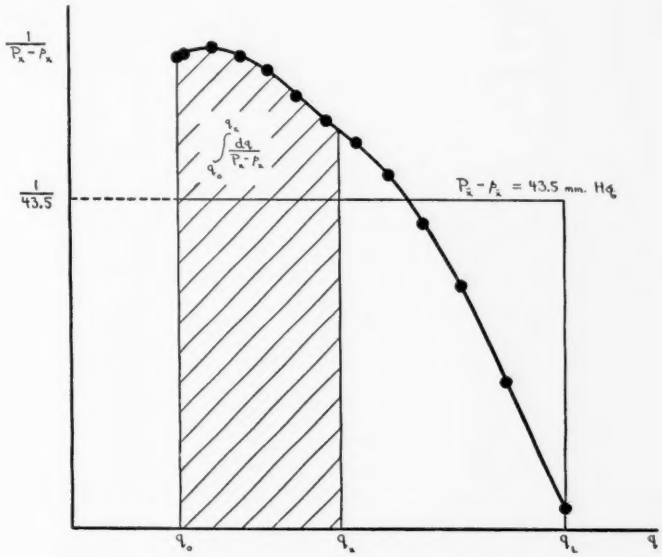
$$\frac{x}{L} = \frac{i}{100\ k}\int_{q_o}^{q_x}\frac{dq}{P_x - p_x}.$$

If the integral is evaluated, we can show how the oxygen tension varies in both the maternal and fetal capillaries from the arterial to the venous ends. To evaluate the integral, we need the actual maternal and fetal blood dissociation curves for oxygen, as determined in the laboratory, and the relationship between q_x and Q_x already derived. Of course, the oxygen content of arterial and venous maternal and fetal blood are also required. To illustrate the procedure, we utilize the data reported in 1952 by Barron and Alexander[4] for sheep ♯5, recomputing per cent oxygen saturation values from smoothed dissociation curves.

For each arbitrary value of q_x between fetal artery and vein, we compute the corresponding values of p_x (from the fetal blood dissociation curve), Q_x (from the formula for it just derived above), P_x (from Q_x and the maternal blood dissociation curve), and so $1/(P_x - p_x)$. Since the integral to be evaluated represents the area under the curve formed from $1/(P_x - p_x)$ as a function of q_x, we measure this area for successive values of q_x in Figure 2, which shows the needed relationship for sheep ♯5. The data and computations derived from them are summarized in Table 1.

OXYGEN TENSION IN MATERNAL AND FETAL SHEEP CAPILLARIES

Since x/L, relative distance along the placental capillaries from the fetal arterial to venous end, is proportional to the integral which has just been evaluated from Figure 2 in Table 1, we show in Figure 3 the functional relationship between P_x and p_x, the oxygen tension in maternal and fetal blood, and our measure of capillary distance (x/L), the integral. Here we see how rapidly oxygen pressure falls in maternal capillaries as the



Maternal	Venous	Q_o	= 9.00	volume	% O_2
"	Arterial	Q_L	= 19.12	"	" "
Foetal	Arterial	q_o	= 1.84	"	" "
"	Venous	q_L	= 8.45	"	" "

$P_x - p_x$ = 43.5 mm. Hg O_2 pressure

FIG. 2. The area under the curve expressing the relationship of $1/(P_x - p_x)$ to q_x gives the value of the required integral, which is proportional to distance along fetal capillaries. The value of $1/(P_x - p_x)$ which gives a rectangle based on q_o to q_L having the same area as that under the curve on the same base is shown. It gives the integrated mean O_2 diffusion pressure for the placenta of the sheep under consideration as 43.5 mm. Hg.

blood first enters from maternal arteries to meet the emerging fetal blood. Later, we shall determine quantitatively the advantage of having maternal and fetal blood flow in opposite rather than the same direction through their contiguous vessels.

TABLE 1. DATA AND CALCULATIONS ON BLOOD SHEEP #5, FETUS 120 DAYS OF BARRON AND ALEXANDER[2]

$\frac{x}{L}$ Relative distance along capillaries from fetal arterial end in % of total capillary length	q_x Fetal O_2 content vols. %	Actual, opposite direction of flow		Hypothetical, same direction of flow	
		p_x Fetal O_2 tension mm. Hg	P_x Maternal O_2 tension mm. Hg	p_x Fetal O_2 tension mm. Hg	P_x Maternal O_2 tension mm. Hg
0.0	1.84	8.0	44.0	8.0	100.0
2.6	1.98	8.8	44.7		
11.2	2.44	11.0	46.6	11.0	87.0
20.2	2.92	12.6	48.6		
28.8	3.39	14.0	50.6	14.0	72.5
37.6	3.88	15.0	52.8		
46.3	4.38	16.0	55.0	16.0	63.2
55.2	4.90	17.0	57.2		
64.1	5.45	18.0	60.0	18.0	57.0
73.2	6.03	19.0	64.1		
82.4	6.68	20.0	69.8	20.0	51.6
91.8	7.44	20.9	80.0		
100.0	8.45	22.0	100.0	22.0	44.0

DIFFUSION CONSTANT OF THE PLACENTA OF THE SHEEP

If we solve our previous equation for k, the diffusion constant in cc. O_2 diffusing across the placenta per minute per mm. Hg O_2 pressure difference, letting $x = L$, we have:

$$k = \frac{i}{100} \int_{q_o}^{q_L} \frac{dq}{P_x - p_x}.$$

To evaluate this expression, one can substitute the area for the integral when q_x is q_L in Table 1, taking care to use correct rather than arbitrary units.

For i we have, by the Fick principle:

$$i = \frac{100m}{q_L - q_o}.$$

Since there is interest in determining that particular point along the placental circulation at which the O_2 diffusion pressure gradient, if constant throughout, would permit the same rate of oxygen transport as actually occurs, we shall determine k by finding this particular value of x, $\bar{x}$. The corresponding value of the diffusion pressure $(P_{\bar{x}} - p_{\bar{x}})$ is called the integrated mean diffusion pressure gradient.

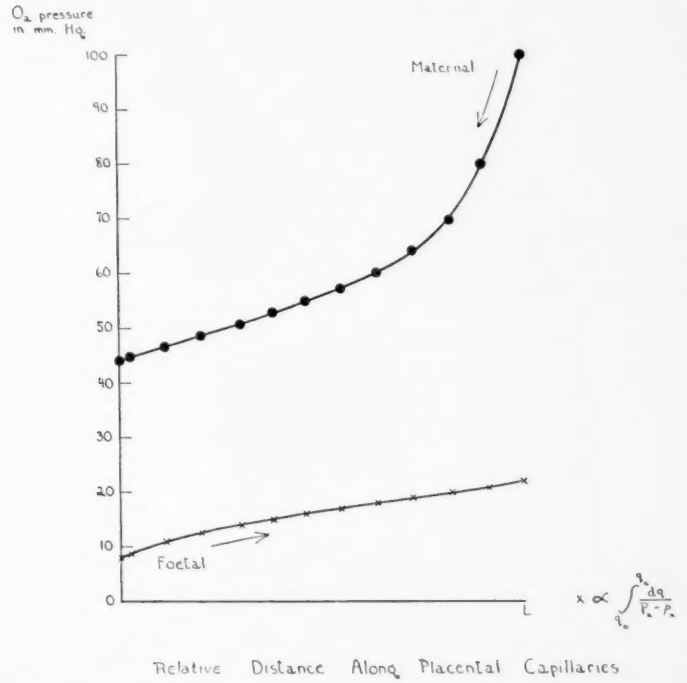


FIG. 3. The changes in O_2 tension in maternal and fetal blood in the sheep's placenta as a function of relative distance along the fetal capillaries are shown. Relative capillary distance is taken as proportional to the area under the curve shown in Figure 2, while the corresponding values of O_2 tension (both P_x and p_x) from which this curve was drawn are shown as ordinates.

In Figure 2, we find that single value of $1/(P_x - p_x)$ which will give a rectangle based on q_0 to q_L having the same area as that under the curve for the same base. $\bar{x}$ is found to be 0.67L. This is to say that two-thirds of the way towards the oxygenated end of the fetal stream the diffusion gradi-

ent equals the integrated mean value. At this point, the fetal blood has acquired about 60% of the oxygen it will finally receive in its passage through the placenta. The value of $P_{\bar{x}} - p_{\bar{x}}$, the integrated mean diffusion gradient, is 43.5 mm. Hg O_2 pressure difference. It is used to determine k:

$$k = \frac{m}{43.5.}$$

Using Barcroft's figures, as quoted by Barron and Alexander[2] for the oxygen consumption of a sheep fetus at 110 days, or 4.2 cc. O_2 per min. per kilo,* and allowing for a fetal weight of 1300 grams, we estimate m at 5.5 cc.O_2/min.

In this way, we obtain for the diffusion constant of sheep #5:

k = 0.126 cc./min./mm.Hg O_2 pressure difference.

This is essentially the same as the value reported by Barron and Alexander.[2]

DIFFUSION COEFFICIENT REQUIRED TO TRANSPORT OXYGEN IN SHEEP IF MATERNAL AND FETAL BLOOD FLOWED IN SAME INSTEAD OF OPPOSITE DIRECTION

Here we assume the same concentrations of oxygen in artery and vein of fetal and maternal supply, only considering the effect of a reversal of flow direction of maternal blood so that in both sets of vessels blood flows in the same direction. The expression for $\frac{dq}{dx}$ is unchanged, but now the sign changes for $\frac{dQ}{dx}$:

$$\frac{dQ}{dx} = -\frac{100k}{IL}(P_x - p_x).$$

The result is:

$$Q_x = Q_o - (q_x - q_o)i/I.$$

Our evaluation of $\frac{x}{L}$ remains:

$$\frac{x}{L} = \frac{i}{100\,k}\int_{q_o}^{q_x}\frac{dq}{P_x - p_x}.$$

But the evaluation of the integral is changed by the new expression for Q_x. Otherwise the procedure is the same. Table 1 gives the pertinent data

* Sheep #5 is 120 days old, but at 136 days the fetal consumption is 4.3 cc., so that the 4.2 cc. figure still applies here.

and Figure 4 shows the graph of $1/(P_x - p_x)$ against q_x to evaluate the integral. The result is that $\bar{P_x} - \bar{p_x}$, the integrated mean diffusion gradient, becomes 39.2 mm. Hg, the steady pressure available (other conditions unchanged except diffusion constant and flow direction) to move the required oxygen supply. Such a gradient, to be effective, requires a diffusion constant of 0.139, 11% higher than the one actually prevailing with opposing direction of flow. Clearly the opposing direction of flow of maternal and fetal blood is the more efficient possibility.

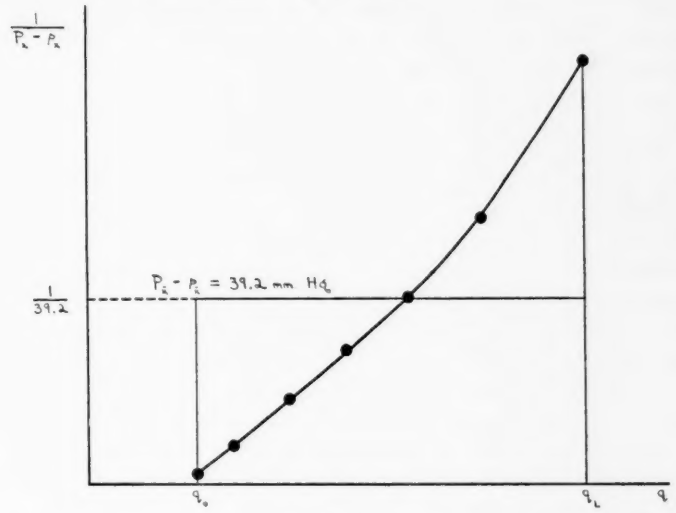

1/(P_x - p_x)
1/39.2
P_x - p_x = 39.2 mm Hg
q_0
q_L
q
Volume % O_2 of Foetal Blood in Placental Capillaries if Foetal and Maternal Blood Flowed in Same Direction in Adjacent Capillaries
K required is 0.139 cc./min./mm Hg O_2 difference
This is 11% more than 0.125 actually found

FIG. 4. This curve is derived exactly like that of Figure 2, except that the assumption is made that the maternal blood flow direction is reversed, so that both fetal and maternal blood flow in contiguous vessels in the same direction. The initial and final values for oxygen tension in maternal and fetal blood are assumed not to have changed. The result is that the integrated mean oxygen diffusion pressure available for transport of oxygen to the fetus falls to 39.2 mm. Hg; a diffusion constant 11% higher than the actual one would be needed if the two circulations flowed in the same direction.

It is interesting that blood and water also flow in opposite directions in the gills of fish. Hazelhoff and Evenhuis[4] found that oxygen utilization dropped from 51% to 9% when the water flow direction was experimentally shifted to the same direction as blood flow.

SUMMARY

Oxygen diffusion in the placenta of sheep has been analyzed, with a numerical example from data in the literature. The change in oxygen content of fetal and maternal blood in their course through the placenta has been described in tabular and graphic form and the method for finding the diffusion constant of the placenta illustrated.

If maternal and fetal blood flowed in the same direction rather than counter-current in contiguous vessels, it has been shown that the diffusion constant in the example chosen would have had to be 11% higher. The actual opposed flow direction is therefore more efficient than the other possibility.

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EPIDEMIOLOGY AND ITS TEACHING IN MEDICAL SCHOOLS

Few textbook descriptions of any common or important infectious disease would be considered adequate today if a section on its epidemiology were not included, and this statement can now include a number of noninfectious diseases. Thus, whether or not we wish to regard the subject of epidemiology as a *basic* medical science, it has come to occupy an *essential* position today in modern medicine. This position lies perhaps midway between pathology, microbiology, public health and clinical medicine, with special implications with regard to preventive medicine. And, if preventive medicine is to be the medicine of the future, here is another reason for recognizing the coming of age of epidemiology. Why, then, is this subject not taught more often to medical students?* I raise this question without attempting to answer it here and now. It may be that the teaching of epidemiology per se has not yet come of age. Or it may be too advanced a subject, too specialized, to be digestible for undergraduate students; or it may be that it merely has appeared on the scene too late to be sandwiched into a curriculum already crowded with expanding old subjects and new subjects clamoring to be taught. Indeed, if all the newer concepts or developments of modern medicine were to be squeezed into this curriculum, the student's day would have to be increased by many hours, and his head might soon reach the bursting point. So, if there are pros and cons to this situation, it at least offers a point for discussion. It would be unwise to begin this discussion, however, without first giving a definition of epidemiology, and a definition of what I shall term *clinical epidemiology* so that the reader may appreciate what we are talking about. It is the latter discipline which has growing implications in the teaching and practice of modern medicine.

If we look in older medical dictionaries, we find the term "epidemiology" defined as "the sum of what is known regarding epidemics." But such a description has now been supplanted; I believe that it reflects the thinking of an earlier generation—at least definitions recently coined by epidemiologists in this country and in this generation interpret their subject with

* A canvas of teaching in epidemiology in the medical schools of the United States and Canada is currently in progress. Preliminary indications point to a dearth of courses which are formally designated as *epidemiology*, although parts of this subject may be taught under other titles in many schools.

much more liberality.* Thus we are no longer limited, in our thinking here, to the concept of epidemics or even to the concept of infectious disease. Our own simple definition indicates that modern epidemiology is concerned with the *circumstances under which diseases occur: where they tend to flourish and where they do not.*

This broader meaning does not subject the word to too much stress, for its derivation, coming as it does from *epi*—on or upon, plus *demos*—people, plus *logos*—treatise, connotes the basic fact that epidemiology treats with something that has been thrust *upon* the people, a something which might be a smog or a heat wave, but which does not have to be an infection. A related term is "endemiology," which I do not find in the dictionary and which might imply something—like bad habits—already *in* the people. An important implication here is that we are dealing with something, presumably undesirable, which affects more than one person. In other words, we are dealing with a blighted population, whether that population be large or small. But whatever one wishes to call it, our concept of epidemiology implies that disease is more than a personal affair. In its study we become concerned with the mechanism of the occurrence of human blights and how the factors responsible for them may be measured.

Thus all kinds of human blights, all kinds of diseases—cancer, benzol poisoning, alcoholism, drug addiction, automobile accidents, and suicides—have their epidemiology or endemiology, or at least they should have. It may be trite to add that an important reason for engaging in this study rests on the fact that if epidemiology is concerned with the circumstances under which disease occurs, then before one sets out to alter these circumstances in an effort to *prevent* disease, it is well to know beforehand what they are and what their significance may be. In this fashion epidemiology is the backbone of preventive medicine.

According to some, notably Professor Gordon[4] of the Harvard School of Public Health, the concept of epidemiology is essentially one of medical ecology. This is mentioned at the risk of our becoming entangled with more

* In Smillie's textbook of *Preventive medicine and public health*[12] modern definitions of epidemiology were as follows:

Epidemiology has been defined by C. O. Stallybrass as "the science which considers infectious diseases—their courses, propagation, and prevention." This definition considers the field of epidemiology in its narrowest sense. Dr. W. H. Welch, once called epidemiology "a study of the natural history of disease."

A more comprehensive and modern conception of epidemiology was expressed by W. H. Frost: "It is the science which considers the occurrence, distribution, and types of diseases of mankind, in distant epochs of time, at varying points on the earth's surface; and secondly, will render an account of the relations of these diseases to inherent characteristics of the individual, and to the external conditions surrounding him and determining his manner of life."

definitions, but it would appear that the use of the term "ecology" here has the advantage of implying broadly that this description embraces the struggle for survival of living things, whether they be tubercle bacilli, plants, or animals, or man. But it introduces a biological rather than a clinical slant to our subject, and so in offering this point of view, one should decide whether one wishes to approach this subject as a biologist or a clinician. In the latter connection it should be recalled that disease in man is not a bacterium, or a virus, or a poison; neither is disease a lesion, or disturbed physiology, or a set of symptoms, or a clinical picture; it is a summation of many features which produce illness in one or more individuals. Disease or disability is thus a process, in which it is convenient to visualize a single cause, but usually there are a variety of causes, a few of them familiar, others quite obscure. In an effort to illustrate this point, we can turn to an old agricultural simile—diseases, like crops, are dependent upon the *seed,* the *soil,* and the *climate.**

The seed represents to most of us something that is tangible. It is often designated as *the* cause, or *the* etiological agent, whether it be a virus, a bacterium, a plasmodium, or a chemical or physical agent, or even something of a psychic nature, such as anxiety or fear. It probably still deserves a top priority among causes of disease, for it is indeed satisfying to regard the seed as an entity, something that can be dealt with, isolated, crystallized, or perhaps put into a test tube and measured. Only so can it have a satisfying meaning to a scientist.† This is helpful, too, for teaching purposes, because it is an attempt at simplification of a complex business. Nevertheless, it is an oversimplification of the epidemiologist's problem if one attempts to confine one's self to the seed alone. For, even with the subject of infectious disease, it becomes abundantly clear that microbes or parasites are only a part of a disease process that goes far beyond the concept of the seed. Microbiology is not epidemiology, nor has it been since Pettenkoffer's day. Thus, in tuberculosis one has almost never regarded the tubercle bacillus as being the one and only cause of that disease; it is a major cause in a situation where host resistance and environmental conditions play important rôles in determining the acquisition or the severity, of the clinical infection. In pneumococcus pneumonia, a given type of pneumococci may not actually be as potent a cause of this illness as is February weather. And

* Gordon[4] has recently paraphrased these factors in reverse order in terms which also date from biblical times, i.e., "the world, the flesh and the devil."

† ". . . new knowledge is not knowledge at all unless it is precise and verifiable, . . ." a quotation from a recent address by H. S. Gasser before the Association of American Physicians at their annual meeting in Atlantic City, N. J., May 2, 1954.

when it comes to noninfectious diseases such as peptic ulcer or coronary occlusion, we are often in greater difficulties if we try to consider the situation in the light of a single measurable cause.

In all of these conditions, man's resistance both from an hereditary and environmental background occupies a dominant position as regards the diseases to which he is liable to fall heir, and so this brings us quickly to a consideration of the next item, the *soil,* which, according to our agricultural simile, is the condition of the host. To the clinician, so-called host factors represent man's resistance and his ability to cope with or succumb to infection, injury, or insult. Man's vulnerability is much more difficult to measure or titrate than is the "seed" in terms of dosage; and vulnerability is often impossible to deal with therapeutically in the same manner as one might treat a bacterial infection with an antibiotic, or a case of poisoning with BAL. For this reason, therapeutists often maintain an exaggerated view of the importance of the seed in comparison to that of human resistance. For it is only natural to emphasize the things that we can comprehend or handle rather than the things we do not know and cannot measure, much less comprehend. Nevertheless, the use of skin test (Schick and tuberculin tests) surveys, and the new approaches to *serological epidemiology* do represent measures of man's resistance; and, therapeutically, the use of immunization procedures, vitamines, and hormones, shows how one does "treat the soil."

A third factor is the *climate,* which is the environment to which both seed and soil are exposed. Obviously, there is much that cannot be measured here also. In fact, it has been said that epidemiology has no more right to be considered an exact science than has meteorology. Both deal with nebulous things. But, at least epidemiological climates can be divided into two parts, or they have been, i.e., so-called macro-climate and micro-climate. Macro-climate is climate in the ordinary meteorological sense, i.e., temperature, rainfall, humidity, etc. Micro-climate is the sum of those intimate living conditions, socio-economic or domiciliary conditions in which a given individual finds himself and which affect his exposure to illness and his resistance against it. It includes those actual details of rural or urban living: poor housing, dampness within the home or working place, crowding, environmental sanitation whether poor or good, inadequate food, and all the circumstances of poverty or affluence. For example, poor housing and crowding can become causes of upper respiratory infections, even more consistently perhaps than are viruses or pneumococci; likewise, the aftermath of war and all its attendant conditions create a climate in which famine and epidemic typhus arise.

Even though it is difficult to measure and analyze all these circumstances, one thing is clear, namely, that we are about to graduate from an analytical type of philosophy, which expects a single etiological factor to be the cause of most clinical entities. It is a willingness to recognize multiple factors of varying importance and their interrelationships that is needed here. Such a pattern calls for a synthesizing point of view. Host resistance and climates, whether macro- or micro-, belong within this pattern. If charted, the scheme resembles the pattern of a nomogram in which, if one point is moved, all other points are affected.*

To turn next from definitions to methods of study, let us begin with an example of what such a pattern could be in a small population and how it could be applied. One can turn first to the subject of the spread of a disease through a given family. This idea of selecting and considering the family as a unit through which disease may spread has considerable justification. Its value lies in the fact that common hereditary and environmental conditions exist in a group of individuals living in intimate contact with one another, who, for a certain period of time at least, are quite conscious of their group life. The family thus becomes an ideal group to which the clinical epidemiologist can turn. Starting usually with a single member of the group as his patient, he can turn to the setting in which that patient became ill and bring his judgment to bear not only on the patient's illness, but upon the situation. The use of a family chart in the form of a linear diagram which relates the number of members in the family, their ages, and the relationship of one illness to another in point of time, has been of help in this type of study. Such a chart is in itself a type of nomogram. It was employed in principle a generation ago by psychiatrists in the form of a graphic life chart on which were listed hereditary, growth, and environmental factors which might have had some bearing on mental health.[9] The amplification of the life chart of an individual to the life chart of a family was only a step, taken in the 1920's by Dr. Opie and his collaborators in their studies on the spread of tuberculosis through families.[7] The Departments of Pediatrics and of Internal Medicine of the Yale University School of Medicine have used modifications of such charts for more than twenty-five years in recording events in a variety of infectious diseases such as scarlet fever, rheumatic fever,[10] poliomyelitis, and respiratory infections. Often the chart of the family has become an adjunct to the hospital history, for a diagrammatic

* For a description of nomograms originally devised by O'Ocagne and subsequently used by many, including L. J. Henderson, see Northrop, F. S. C.: *Science and first principles.* New York, The Macmillan Co., 1931, pp. 187-205.

chart is much better for this purpose than a long, written history. The type of chart which has been employed is perhaps best illustrated by a sample diagram (and its explanation) one of which appears in Figure 1. Here is depicted a "case" seen in the New Haven Hospital some twenty-five years ago which can be regarded as a simple case of lobar pneumonia in one sense,

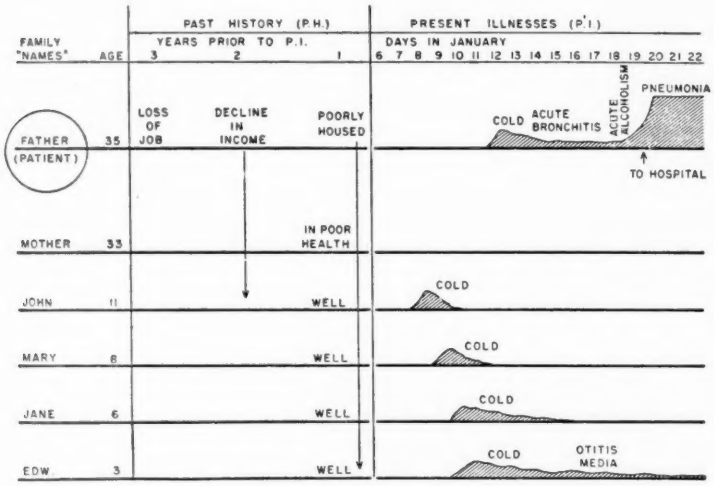

FIG. 1. A case of pneumonia in an adult, 35 years of age, and its relation to a few of the preceding events in the life of the patient and the life of his family. The six heavy horizontal lines indicate the six members who compose the family group. Their names and ages appear at the left side of the diagram. The patient appears on the top line as the male parent of this family. A few medical events have been recorded chronologically on this chart (as a rule it is simpler to indicate these with a series of legends which stand for different illnesses). The major points are that one can see at a glance the size of the family group, the general character of the health of its members, the relationship which certain events bear to illness and which multiple familial illnesses bear to one another. It culminates in a major illness in an individual who, either through bad luck or bad management, was perhaps in a somewhat vulnerable position for the contraction of pneumonia, and in whom the physician's responsibility would seem to be more than that of treating pneumonia alone.

but as a complicated case in another (epidemiological) sense. To illustrate the two points of view let us consider the pathogenesis of this case of lobar pneumonia in an adult of thirty-five years of age. As far as the practitioner of curative medicine is concerned this case of lobar pneumonia is an acute and serious illness caused by the pneumococcus. The pneumococcus is the only etiological agent with which he is immediately concerned, because by

bringing to bear his knowledge about this agent, he can proceed at once to a rational method of treating the patient. To the clinical epidemiologist, the case of lobar pneumonia is still a pneumococcal infection but besides this he may wish to consider how this infection came about (or at least how it was conditioned) by a long train of preceding events which may date back several years such as, for instance, the patient's loss of employment three or four years previously; his subsequent growing tendency to chronic alcoholism and vagrancy; his neglect of his family of four children who are now housed in poor and damp living quarters; his recent exposure to a familial epidemic of colds which started among the children, one of whom now has acute otitis media, his own contraction of a cold; his attempt to cure it with alcohol resulting in a night on a park bench, and pneumonia. According to this point of view the pneumococcus brings up the rear in a long chain of events; it is a secondary invader so to speak, almost a final invader.

It is easy to see how it is only another short step from the family to a small community and how the country doctor has some advantage here, an advantage which goes back for centuries. An example is our own Nathan Smith, who watched the comings and goings of typhoid fever in the early nineteenth century in the towns and villages throughout Connecticut, which he had come to know so well.[8] In the twentieth century, the number of such doctors declined, but there is no better recent example of this sort of practice than that to be found in Dr. Pickles' book entitled, *Epidemiology in country practice*,[11] describing his practice in twentieth century rural England.

It would be a mistake at this point to suggest that the task of the epidemiologist consists essentially of observation of patients and their contacts, the rates at which they acquire diseases, and the mere charting of this situation. Actually, as in other clinical sciences, it depends on accurate measurements of one kind or another, their orderly arrangement, and thoughtful consideration. Certainly, the introduction of a critical attitude with regard to statistics and their application here has been responsible for the development of the subject of epidemiology as a would-be science in its own right. In few clinical fields or public health fields has the value of bio-statistics come to the fore more quickly than in the practice of epidemiology and its investigation. British epidemiologists[5, 12] recognized this quite early; and in this country it was largely due to the efforts of Professor W. H. Frost, during the 1920's and 1930's at the Johns Hopkins School of Hygiene, that something was done to transform epidemiology from a speculative type of discipline, into one more worthy of the name of medical science.[3] However,

the type of epidemiology which should be taught to medical students is not an abstract discipline, it is not statistical epidemiology per se, it is a combination of clinical and statistical techniques. For neither the statistician nor the clinician can develop the field of epidemiology alone. The statistician's usefulness is obvious, but the value of his analyses are dependent upon the accuracy with which his data are collected and upon a knowledge of the manner of their collection. The clinician's contribution is less easy to define but he should decide what is or is not important in the situation. A combination of these talents together with those of the laboratory, has given rise to a new clinical science which has been termed *clinical epidemiology*.[2, 9]

Indeed, the point to be made here is that the clinician stands in relation to the statistical epidemiologist perhaps as a gardener does to a farmer. They both dig in the ground, but one does it on a larger scale and with machines, the other by hand. The statistician increases the significance of his analyses by increasing the numbers of observations, whereas the clinician can hardly hope to expand this way; instead he has the opportunity of improving the accuracy of his data with intimate and exacting observations and the development of clinical judgment. This restriction of the size of the group with which he deals rests on the fact that clinical talents *cannot be applied wholesale* without the risk of their being spread too thin to be effective. Fortunately or unfortunately, the amount of personal attention requisite for the exercise of clinical judgment is set for most physicians by physiological limits which cannot be easily exceeded. In the ordinary practice of medicine, and in our efforts to understand disease, we are constantly called upon to practise epidemiology and examine circumstances intimately and this calls for as much diagnostic acumen as does physical diagnosis and good history-taking. It means that it is not only the physician's duty to examine the *patient* according to the usual methods of physical or laboratory diagnosis, but also to record and examine carefully the *circumstances* under which the patient became ill—and what is very significant—under which he is likely to become ill again. Here is the fundamental contribution which clinical epidemiology has to make to preventive medicine in a busy age of multiple injections given by a physician who spends much of his day shuttling back and forth from office to hospital with scant time for reflection.

An example of this sort of detective work can be found in the story of Zenker and trichinosis, which has been told so well by Blumer.[1] Zenker noted the relationship between the lesions in human trichinosis and those in the pig by the study of one small outbreak starting with a single patient.

The story is one worthy of *The New Yorker*. It is like that of a detective visiting the site of a crime. Zenker's success was the result of an interest in details under which illness arose and his willingness to go out of the hospital into the setting where his patient became ill. It was his aim thus to place the patient in the pattern which gave rise to illness rather than to regard him as a lone sick individual who suddenly popped out of a healthy setting.

If instruction in epidemiology is to be given in medical schools, the question may be properly raised as to where it belongs in the curriculum. Before attempting to answer this question each school should decide whether the subject deserves to be regarded as a minor medical specialty (for which an elective course in the third or fourth year would probably be the answer) or whether it deserves to be considered as a major subject aimed to condition the thinking of the medical student throughout his entire medical career, regardless of what branch of medicine he enters. If it belongs in the latter category, and the student is to be exposed to the subject throughout all four years of his medical course, then it would be well to make enquiry of various departments of the medical school, such as microbiology, medicine, pediatrics, and others, to determine how much or how little in the way of epidemiological concepts is already being taught in these departments. Certainly in microbiology there is an opportunity to introduce the subject of epidemiology in the first or second years; and in a number of medical schools in this country, courses or lectures in epidemiology either accompany or follow those of microbiology. That is a useful way to "sensitize" students to this subject.

However, in the present report, emphasis has been laid upon what we have called *clinical epidemiology* which, like other clinical subjects, can best be taught in the clinical years. It has been apparent for some time that third- and fourth-year medical students are more interested in sick people, and in learning how to become an interne, than in abstract subjects. For this reason we have found it advisable to organize the teaching of clinical epidemiology as a bedside or dispensary discussion. This can be done with small groups concerned with patients whom one or more students already know. The discussion starts with the illness and is then gradually led away from the bedside. The object here is to develop an interest in a sphere which lies outside the immediate care of a hospitalized patient. Sometimes this approach appears beside the point to the student, who only wants to know how he can learn to cure his patient. Specifically the teacher is called upon in these discussions to consider the accuracy of the diagnosis and the various diagnostic possibilities, then to point out to the student the value of

making a more complete review of the patient's past history than one usually finds in the average hospital record in an effort to find what causal factors exist. This more adequate family history should be taken with particular reference to the circumstances under which the patient has lived and should include an occupational history. The value of conversations with members of the patient's family is stressed, together with the value of studying past hospital records and the records of other members of the family. When possible, a visit by the student to the patient's home is advised. He is also asked to consult with the Social Service Department whose members can review the situation from present and perhaps past angles. In this fashion the necessity for building up the picture of the disease and the setting in which it was acquired is brought to the student's attention. Subsequently, and here one goes over to *applied* epidemiology, the attempt is made to predict how the situation which led up to the patient's illness might have been altered, and what the future holds for the patient when he returns to his original environment.

In the fourth year a series of lectures can be given, which are aimed to fill in gaps which one cannot hope to cover in a bedside or dispensary discussion. Here a number of important diseases can be presented from the angle of their epidemiology, such as tuberculosis, venereal disease, tropical disease, disease in animals which can be transmitted to man, coronary heart disease, and rheumatic heart disease, malnutrition, etc., with a view to analyzing the circumstances under which they occur and how the circumstances might have been altered. Obviously, this type of applied epidemiology almost automatically leads directly into preventive medicine.

This, then, may give us some idea of what is meant by epidemiology and some of the reasons why it has come of age and why *clinical epidemiology* is that part of the discipline which, it would seem, would be particularly pertinent for medical students.

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INCREASED SUSCEPTIBILITY TO SHIGELLA ENDOTOXIN AND TO HISTAMINE AFTER IMMUNIZATION TO H. PERTUSSIS

Acquired sensitivity subsequent to vaccination is a significant problem in prophylactic medicine, and the observations which are to be described seem pertinent to this problem. These studies concern immunization with *Hemophilus pertussis* vaccine which renders mice extremely susceptible to subsequent injections of the same vaccine as well as other bacterial preparations and histamine. It is, therefore, possible to compare the susceptibility of normal mice of different ages to *H. pertussis* vaccine and after vaccination to challenge inoculations of the various preparations. Histamine has been included because of its repeated link with sensitivity reactions even though its rôle has not been rigidly established.

In utilizing the technique of altered susceptibility through pertussis vaccination it has been found that older mice are much less able to withstand an initial injection of the vaccine than are younger animals. Once vaccinated, all mice pre-treated with pertussis have a markedly decreased resistance to a subsequent inoculation of *Shigella* endotoxin. In an attempt to isolate the responsible fraction, mice have been tested with purified preparations of polysaccharide from *Shigella dysenteriae*.

MATERIALS AND METHODS

A complete procedure for sensitization with *H. pertussis* has been presented elsewhere[6–11] and only the total dosage and routine of injections will be included in the description of the particular experiments.

The mice used in these studies were strain CFW and formed two major groups: old ex-breeders more than one year of age, weighing 30-32 gm., and young mice less than one month old, weighing 18-19 gm. Most experiments were with female mice and when males were used, a special note is made. In our experiments we used the "O" antigen polysaccharide of *Shigella dysenteriae*, kindly supplied to us by Dr. H. P. Treffers of this Department. It was prepared by the extraction of *Shigella dysenteriae* according to the phenol method of W. T. J. Morgan. Two preparations were required and are designated in the tables below as lots #82 and #83.

Received for publication July 1, 1954.

OBSERVATIONS

Vaccination with pertussis and the effect of age. In these studies the age and sex of the mice were always considered. In the first experiment old mice, ex-breeders more than one year of age, and young mice less than one month old were all injected intraperitoneally with 15 billion cells of *H. pertussis* vaccine in a single dose. Only 57 of 96, or 59%, of the older mice survived in comparison with 112 of 117, or 97%, of the younger group, death occurring from 4 to 12 hours after the injection. The difference between young and old is statistically significant with $p = 0.01$. When young mice were injected with 30 billion cells of the pertussis vaccine in two intraperitoneal doses of 15 billion cells each, given one day apart, 46

TABLE 1. SENSITIVITY TO HISTAMINE PRODUCED BY H. PERTUSSIS VACCINE IN DIFFERENT AGES OF MICE

	Old ex-breeders	*Young mice*	
Dose of histamine mg. per kilo body weight	*15 billion cells H. pertussis*	*15 billion cells H. pertussis*	*30 billion cells H. pertussis*
25 mg.	9/26	5/6	
50 mg.	3/23	10/10	2/6
75 mg.		3/3	
100 mg.		6/7	0/5
200 mg.		3/4	

of 54 animals, or 85%, survived. Thus, it is shown that *H. pertussis* vaccine is much more toxic for older mice than for young animals of the same strain.

Susceptibility to histamine after pertussis vaccination. Survivors from the preceding experiment, having been inoculated with *H. pertussis,* were given an intraperitoneal injection of histamine five days later. The data are included in Table 1. According to previous published experiments[8] histamine is not toxic for young normal mice in doses of 2,000 mg./kg., but is toxic for pertussis-treated animals in doses less than 50 mg./kg. Old ex-breeders which had survived the injection of 15 billion cells of *H. pertussis* vaccine given in a single dose were much more susceptible than treated mice of a younger age. Death occurred 4-12 hours after the injection of histamine. In the older group, 9 of 26, 35%, survived a dosage of 25 mg. of histamine/kg. and only 3 of 23, 13%, withstood 50 mg. of histamine/kg. By comparison, young mice which were relatively unaffected by the injection of 15 billion cells of *H. pertussis* vaccine were better able to withstand the challenge of histamine, 3 of 4, or 75%, surviving a dosage of 200 mg./kg. and 23 of 26,

or 90%, surviving smaller doses. The difference in susceptibility to histamine between young and old mice treated with pertussis is quite significant statistically with $p = 0.01$. However, when young mice were inoculated with 30 billion pertussis organisms injected in two equal doses on two consecutive days, none of 5 survived a dosage of 200 mg. of histamine/kg. The difference in susceptibility to histamine between young mice pre-treated with 30 billion cells and those pre-treated with 15 billion cells is also of statistical significance ($p = 0.01$).

Susceptibility to Shigella endotoxin. Older animals can be pre-treated with *H. pertussis* with a mortality of approximately 10% by a procedure

TABLE 2. SUSCEPTIBILITY OF NORMAL AND SENSITIZED MICE TO SHIGELLA ENDOTOXIN, LOT 82

Dose of Shigella *toxin per mouse*	*Ex-breeders, female, sensitized with* H. pertussis *vaccine* (*a*)	*Ex-breeders female normal* (*b*)	*Ex-breeders male normal* (*c*)	*Young female normal* (*d*)
1.0 mg.	0/3	0/5	0/13	1/5 (20%)
0.5 mg.	0/3	1/6 (17%)	9/18 (50%)	1/3 (33%)
0.25 mg.	0/6	2/11 (22%)	11/16 (69%)	7/9 (78%)
0.12 mg.	0/3	6/10 (60%)	2/6 (33%)	2/3 (67%)
0.06 mg.	0/5	4/5 (80%)	4/6 (67%)	2/3 (67%)
0.03 mg.	0/4			3/3 (100%)
0.0075 to 0.15 mg.	0/10			
0.005 mg.	3/6 (50%)			
0.001 to 0.002 mg.	4/4 (100%)			

of injecting 15 billion cells in 3 doses of 5 billion cells each on 3 consecutive days. Ex-breeders, 44 in number, were thus treated and then injected intraperitoneally with various dosages of *Shigella* endotoxin. The results are shown in column a of Table 2. In test doses from 0.0075 mg. to 1.0 mg., not one animal survived; 3 of 6, 50%, survived after the inoculation of 0.005 mg.; all of 4 survived doses of 0.001 mg. to 0.002 mg. When death occurred, it came 4-12 hours after the injection of the endotoxin.

To provide a comparison, 37 normal ex-breeders were also injected with varying dosage of *Shigella* endotoxin (column b of Table 2). All of 5 animals succumbed to a dose of 1 mg.; 1 of 6, 17%, survived an injection of 0.5 mg.; 4 of 5, 80%, withstood 0.06 mg. Thus, the effective challenging dose is approximately 100 times as great for normal animals as for pertussis-treated animals of the same age and sex. The difference between

the normal and vaccinated female ex-breeders is statistically significant ($p = 0.01$) for the dosage ranging from 0.0075 to 0.500 mg.

The preceding experiment has been repeated using a second preparation (#83) of endotoxin polysaccharide, the results being summarized in Table 3. The largest dose which any pertussis-treated ex-breeder survived was 0.015 mg. or 1/27 or 0.4 mg., the largest dose withstood by a normal ex-breeder. In the dosage range of 0.015 to 0.400 mg. this difference was significant ($p = 0.04$).

In comparing the susceptibility to *Shigella* endotoxin as related to sex, 96 normal ex-breeders, 59 male and 37 female, were challenged with vary-

TABLE 3. SUSCEPTIBILITY OF NORMAL AND SENSITIZED MICE TO SHIGELLA ENDOTOXIN, LOT 83

Dose dysenteriae *endotoxin*	*Ex-breeders sens. with 15 billion* H. pertussis *cells* (*a*) *	*Ex-breeder normal* (*b*)	*Young mice sens. with 15 billion cells* (*c*)	*Young mice sens. with 30 billion cells* (*d*)	*Normal young mice* (*e*)
0.0006 mg.	....	....	...	...	....
2.0 mg.	0/4	0/7	0/3		3/13
0.4 mg.	0/4	1/11	0/8	0/2	6/10
0.08 mg.	0/8	4/7	6/9	0/5	4/4
0.015 mg.	2/12	3/4	3/4	1/5	4/4
0.003 mg.	2/7			2/3	
0.0006 mg.	7/7			5/6	

* Fractions indicate number of survivors out of number exposed.

ing dosage of endotoxin (Table 2, columns c and d). The older male mice appear to be slightly less susceptible to the toxin than the normal female ex-breeders ($p = 0.04$ for the dosage range from 0.25 to 0.50 mg.).

In a consideration of age, it was shown (Table 3, columns b and e) that normal young mice are more resistant to the *Shigella* polysaccharide than normal old ex-breeders, since the latter succumbed more readily to the test doses ($p = 0.01$). However, by using a large inoculum of pertussis vaccine to pre-treat these young mice, their ability to withstand the inoculation of endotoxin could be markedly reduced ($p = 0.02$ for 0.015 to 0.080 mg.).

DISCUSSION

The increased susceptibility to *Shigella* polysaccharide which is shown by mice immunized with *H. pertussis* vaccine extends our earlier observations concerning the capacity of this vaccine to induce a non-specific sensitivity

to vaccines of several species of Gram negative bacteria, as well as sensitivity to the homologous antigen.[6–11] The latter observation has been substantiated recently by Kind.[8] These studies would indicate certain significant ramifications concerning the rôle of non-specific sensitization in the resistance and susceptibility to infection. For example, when mice are immunized with *H. pertussis* vaccine they become resistant to infection by the homologous organism but, when compared to normal, non-treated animals, they are found to be more susceptible to infection with other Gram negative bacteria such as *Proteus vulgaris OX19, Pasteurella multocida,* and *Brucella suis.*[12, 14]

One possible explanation of this phenomenon is that the Gram negative bacteria contain a common antigenic substance which has sensitized the animals. That there is an antigen in common is supported by the papers of Clark, *et al.*[5] and White,[15] who have shown a common protein among the *Salmonella, Flexner, Coli,* and *Proteus* organisms. The current experiments are of special interest, however, since a highly purified polysaccharide of *S. dysenteriae* has served as the challenging agent. Furthermore, the use of this purified compound has permitted the more precise measurement of the degree of susceptibility of the treated animals according to age and sex as well as the difference in susceptibility between these animals and normal controls. In some instances the differences was as much as a hundred-fold.

The significance of the increased susceptibility to histamine is more difficult to comprehend. Although the older pre-treated mice are much more susceptible than the younger vaccinated animals, the relative resistance of the young is significantly reduced by more intense pre-treatment with pertussis. While it is known that histamine, when given to normal animals in adequate dosage intravenously, will mimic the species peculiarities of anaphylactic shock and mode of death, its rôle in sensitivity reactions is certainly not established. It would, therefore, be most presumptuous to postulate why pre-treatment with pertussis will lower the physiological threshold to histamine.

The data obtained from these studies may be correlated into several noteworthy facts. Perhaps most important is the observation that older mice are much more susceptible to an initial injection of pertussis vaccine than are the younger animals. This is supported by the work of Kind[8] cited above and by Burky,[1] who showed that young rabbits were not susceptible to a dosage of staphylococcal toxin lethal for adults of the same species. It may well be that this phenomenon is associated with sensitization during life, possibly through the long exposure of adult animals to Gram negative organisms living in the intestinal tract.[10]

A second significant fact is that vaccination to *H. pertussis* will render mice extremely susceptible to a subsequent inoculation of a purified polysaccharide of *S. dysenteriae*. Similarly, pertussis vaccination will increase susceptibility to dosages of histamine otherwise innocuous. Although young mice, pre-treated with *H. pertussis* vaccine were less susceptible to *Shigella* polysaccharide and less affected by the challenging inoculation of histamine, the injection of larger preparatory doses of vaccine markedly diminished the relative resistance of the young.

The rôle of *H. pertussis* in "sensitizing" these animals is certainly not clear and there are related phenomena with pertussis which ought to be mentioned. Malkiel and Kirchheimer[5] induced tuberculin sensitivity in mice by the inoculation of a mixture of BCG and *H. pertussis* vaccine. Similarly, Malkiel and Hargis[4] produced anaphylactic shock in mice by the inoculation of horse serum subsequent to preparatory injections of *H. pertussis* vaccine and horse serum.

In related studies Pittman[15-17] discussed the difference between male and female mice in sensitization to *H. pertussis* vaccine and also reported on the sensitization of mice by intranasal administration of *H. pertussis* culture.

SUMMARY

1. Older mice are much less able to withstand injections of *H. pertussis* vaccine than young mice of the same sex and strain.

2. The injection of *H. pertussis* vaccine increases the susceptibility of mice to injections of *Shigella* endotoxin and of histamine.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. E. Cuyled Hammond and Dr. D. Horn of the American Cancer Society for their expert assistance in the statistical evaluation of the data.

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THE TREATMENT OF REVERSIBLE UREMIA*

Few clinical problems tax the ingenuity, judgment, and patience of the physician as much as does the treatment of the patient with renal decompensation. The inexorably downhill course of patients in the terminal stages of chronic glomerulonephritis is well known. It has been summed up in the words of Page: "It is, I think, a therapeutic achievement when the patient walks into the hospital and dies a few days or weeks after admission."[18] Perhaps understandably, the attitude of the physician called upon to treat such a patient is often tinged with hopelessness and resignation.

It is not widely enough appreciated that even in a patient with advanced nephritis, renal decompensation can be at least partially reversible. Far from being standardized or consisting chiefly of masterful neglect, treatment of the uremic patient should and can be highly individualized, predicated on sound physiological principles, and motivated by a careful and systematic search for those contributory causes of renal decompensation which can be completely or partially reversed by treatment. Table 1 lists some of these causes.

TABLE 1. REVERSIBLE CAUSES OF RENAL INSUFFICIENCY

1. Congestive heart failure
2. Infection
   a. Pyelonephritis
   b. Bacterial endocarditis
   c. Systemic infection
3. Dehydration
4. Hyponatremia
5. Alkalosis
6. Potassium depletion
7. Hypercalcemia
8. Anemia
9. Self-limited acute disorders
   a. Acute tubular necrosis
   b. Acute glomerulonephritis

The chief functions of the kidneys are to remove metabolic waste products and to maintain a constant composition and volume of body fluids. A high level of urea (the most important constituent of the non-protein nitrogen) in the blood, although not in itself harmful, is a useful clinical index of the inability of the kidney to perform these functions properly. As an easily measured index, then, it has an important relation to the functional reserve of the kidneys.

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* Delivered at Noon Clinic, Yale University School of Medicine, March 18, 1954.

Received for publication July 6, 1954.

The quantity of urea (Q) formed and excreted is fairly constant from day to day and is related to the amount of protein metabolized. Under most circumstances, its excretion is in direct proportion to the amount filtered by the glomeruli, and is therefore proportional to the product of two variables:

$$Q = {}_kGFR \times P$$

where GFR = glomerular filtration rate, P = plasma level of urea = concentration of urea in glomerular filtrate, and k is a proportionality constant. Figure 1 illustrates this relationship.

It is apparent that although the concentration of urea in the plasma varies inversely with glomerular filtration rate from the very first reduction in the latter, one-half to three-quarters of both kidneys can be removed before the NPN is necessarily elevated above "normal" limits. Fully two-thirds of both kidneys must be removed before concentrating power is impaired.[19] Conversely, if on a normal diet the NPN is elevated in what appears to be

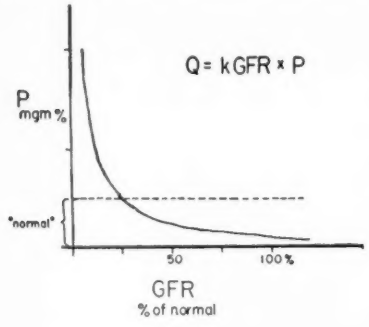

FIG. 1.

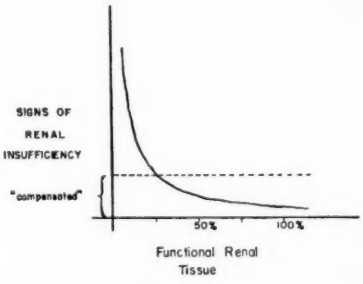

FIG. 2.

unilateral renal disease, the function of the opposite kidney must be assumed to be reduced.

In a very rough way, we may generalize the relationship between functioning renal tissue and the signs and symptoms of renal failure in the same fashion, as in Figure 2. The hyperbolic curve clarifies some aspects of the course of chronic progressive renal disease. One can visualize a slowly progressive process, relentlessly destroying renal substance, which is relatively asymptomatic for many years. When the limits of renal reserve are reached, symptoms appear in swift succession, and the patient goes rapidly downhill, though there may be little actual acceleration of the rate of pathological destruction of the kidneys.

Oversimplified though they may be, these graphs provide a certain background and philosophy for the treatment of renal disease. The less

kidney tissue there is remaining, the more difference a small increment in function makes to the patient. The extent of damage *which is reversible* is therefore tremendously important. The task of the physician in the treatment of advanced renal disease is to salvage every last bit of renal function —every last nephron! A small improvement in function may make the difference between life and death for the uremic patient.

CONGESTIVE HEART FAILURE

A reversible disorder frequently contributing to renal decompensation is congestive heart failure. Digitalization of the patient in heart failure is usually followed by an increase in glomerular filtration rate and renal blood flow, as well as improvement in other renal functions.[7, 8] In patients with apparently normal kidneys, congestive failure may produce albuminuria, casts, and red cells in the urine; however, in such cases the urine is usually of high specific gravity and the NPN rarely over 50-60 mgm. per cent, and practically never as high as 90-100 mgm. per cent.[8] In patients with chronic renal disease, on the other hand, a failing heart may make the difference between renal compensation and progressive uremia.

In chronic nephritis, heart failure is most often secondary to the associated hypertension. In certain instances it may be acutely related to serum electrolyte abnormalities, such as low concentrations of serum sodium or a high serum potassium. In acute glomerulonephritis the reason for the heart failure which is frequently present is not known. It is not clearly correlated with the degree of hypertension and may occur in its absence. With hypoalbuminemia, which is almost universally present to some degree in these patients, edema formation can occur with correspondingly less elevation of venous and capillary pressures. Thus, occasionally a patient may have considerable edema without apparent dyspnea at rest, orthopnea, pulmonary rales, or measurable elevation of the venous pressure, and still be in heart failure. In such a case it may be advisable to digitalize the patient as a therapeutic test. Not infrequently there will be a moderate diuresis and, as edema subsides, improved renal function will be reflected in a falling NPN.

INFECTION

A second important reversible cause of renal decompensation is infection. Active destruction of renal tissue by pyelonephritis may take place without producing fever or shaking chills, leucocytosis, burning or pain on urination, or tenderness in the costovertebral angle. Although these symptoms are usually prominent in an attack of *acute* pyelonephritis, the only clue to the presence of active renal infection may be the presence of white cells and

bacteria in a clean or catheterized urine. Every patient with chronic renal disease deserves a urine culture and treatment with the appropriate antibiotic if an infecting organism is found. The use of inlying bladder catheters should be avoided wherever possible in patients with chronic renal disease. They invariably lead to infection, often with organisms resistant to antibiotics, and may initiate a necrotizing cystitis. In patients wth neurogenic bladders—frequently seen in long-standing diabetes or after poliomyelitis—a stubbornly persistent urinary infection may only be controlled when the bladder is helped to empty completely of its residual urine by the use of parasympathomimetic drugs like prostigmine, urecholine, or furmethide. Superimposed acute infection may occur in any form of chronic renal disease as well as in chronic pyelonephritis, as illustrated by the following example:

> A middle-aged man with polycystic kidneys had been nauseated for a few days and had vomited on the day of admission. On admission to the hospital he was dehydrated, feverish, and somnolent. His urine was grossly infected with paracolon bacillus sensitive to aureomycin. His NPN was 210 mgm. per cent, with a serum bicarbonate of 14 mEq/L. After intravenous saline and glucose he looked a little better and in 10 days specific treatment had cleared his acute urinary infection and brought his NPN down to what for him was a basal 125 mgm. per cent. He was eventually able to leave the hospital and return to his part-time desk job.

In an age of antibiotics, in which *bacterial endocarditis* can be successfully treated, it is important to remember that one of the ways in which this disease can present is as advanced renal failure. The pathological picture is that of a diffuse glomerular nephritis, with focal areas of infarction, proliferation of the glomerular capsule, and capillary thrombi.

Systemic infections—pneumonias and abscesses—as well as infections of the kidney, frequently seem to have a rapidly deleterious effect on renal function in patients with little renal reserve. As a matter of fact, starvation, fever, and injury of almost any type will accelerate the development of azotemia by increasing the breakdown of protein and the load of nitrogenous wastes requiring excretion. Conversely, when these are controlled, the patient may again regain compensation.

OBSTRUCTION

It is important when first faced with a patient with renal insufficiency to consider the possibility of lower urinary tract obstruction. The patient with chronic prostatic retention or urethral stricture, with overflow incontinence and infection that can only be cleared by the draining of a pool of stagnant

urine, may improve remarkably when his bladder is allowed to empty normally. One might predict this from animal experiments in which an acute elevation of ureteral pressure regularly caused a decrease in glomerular filtration rate.[28] Chronic partial obstruction, with hydronephrosis and partial pressure atrophy of renal tissue, results in depression of many renal functions,[14] which is at least partially reversible when the obstruction is relieved. A flat film of the abdomen should be part of the initial workup of every patient with uremia and oliguria, since calculi may obstruct both ureters simultaneously or a single ureter when one kidney is absent. Failure to investigate the possibility of ureteral obstruction in cases like these may be as disastrous as too vigorous instrumentation of the urinary tract in chronic nephritis or acute tubular necrosis.

Patients with obstruction, hydronephrosis, residual urine, and consequent infection frequently do not show the widespread vascular damage, hypertension and circulatory deterioration associated with chronic glomerulonephritis or malignant nephrosclerosis. Partly for this reason perhaps, at the same level of renal decompensation, their prognosis is much more hopeful.

DEHYDRATION

An extremely common sequence of events leading to deterioration of a patient with chronic renal disease is the vicious circle of dehydration. Contraction of extracellular fluids leads to a decrease in glomerular filtration and a diminution in urine flow. This further diminishes the excretory efficiency of an already damaged kidney. Nitrogenous waste products start to accumulate in the blood and the patient becomes slightly nauseated and may vomit. This further depletes the extracellular fluid. Cardiac output may fall. There is a tendency to hypotension. This is accompanied by further depression of renal function, which in turn is responsible for more intractable vomiting and the development of the full-blown syndrome of prerenal azotemia.

Because the patient with chronic nephritis is unable to elaborate a concentrated urine, he must excrete large amounts of urine in order to maintain a constant internal environment in the face of a capricious or indiscriminate appetite. Encouraging these patients to drink is therefore an important part of therapy. In patients who are not edematous this can be done by the judicious administration of salt, preferably in salty foods or drinks. If sodium chloride pills are prescribed, they should be taken with meals to minimize gastric irritation and should not be enteric coated if absorption is to be assured. The salt has two functions. First, it stimulates

thirst, increasing the intake and hence the output of fluids. Secondly, it replaces sodium lost in the urine in excessive amounts by patients with advanced nephritis.

The circle of dehydration, renal insufficiency, vomiting, and more dehydration, can be set in motion by many things. The overnight obligatory polyuria of chronic nephritis can initiate morning dehydration with associated nausea. If not combatted by a carbohydrate drink or a cup of hot milk or coffee upon awakening, a bout of intractable vomiting may ensue. These patients tend to be exceptionally queasy, and drugs or procedures which cause nausea must be used with the utmost caution. Digitalis should not be cavalierly pushed to toxicity. I recall a patient with chronic pyelonephritis who became severely ill because of an ill-advised attempt to convert a chronic auricular flutter with doses of quinidine pushed to the point of vomiting and diarrhea. Similarly, hospital procedures which promote dehydration should not be employed. A breakfast held until bloods are drawn at 10 or 10:30 a.m., or overnight dehydration for a concentration test, may start a serious chain of events in a patient balanced on the brink of renal decompensation.

HYPONATREMIA

In addition to dehydration, with over-all contraction of body fluids, an important cause of progressive uremia which can be successfully treated is hyponatremia,[21] resulting from losses of sodium in excess of water. Patients with damaged kidneys are especially prone to hyponatremia. When active reabsorption of sodium by the renal tubules is impaired, sodium may be "wasted" in the urine, even in the face of a falling serum sodium. This is probably related both to proximal and distal tubular damage and to a greatly increased flow of urine with an increased osmotic load in those few nephrons untouched by disease.[20] "Salt-wasting nephritis" is not a specific type of renal disease, but rather a manifestation of renal insufficiency, regardless of etiology, in which kidneys continue to excrete sodium without proper regard to the serum concentration of this ion.

In patients with or without underlying renal disease the loss of gastrointestinal secretions by vomiting or diarrhea may be an important cause of sodium depletion. The loss of intestinal juices through draining fistulas or constant suction may produce large electrolyte deficits in surgical patients. During profuse sweating, as, for example, on the operating table, the loss of water and salt through the skin may attain significant proportions. The tendency to restrict salt in the postoperative period for fear of excessive salt retention has sometimes resulted in cases of salt depletion with resultant

uremia on surgical wards. Replacement of the fluid lost in such cases with parenteral solutions containing no salt will restore extracellular fluid volume only at the cost of producing hyponatremia, hypotonicity, and overhydration of cells.

Sodium depletion may occasionally be produced by the injudicious use of mercurial diuretics in patients with congestive heart failure, who are eating a diet low in salt. Occasionally, a patient with cirrhosis and ascites may develop signs of the "low-salt syndrome" when following abdominal paracentesis there is a rapid reaccumulation of ascitic fluid. If salt intake is restricted and these patients are allowed to satisfy their thirst with water *ad lib,* hyponatremia, circulatory collapse, and renal insufficiency may develop rapidly.

Regardless of how it is produced, severe hyponatremia per se profoundly disturbs the circulation and the function of the kidneys. As Elkinton, Danowski, and Winkler[8] showed, salt depletion tends to reduce cardiac output and lower arterial pressure. Plasma volume is contracted even though total extracellular fluid volume may remain the same. Glomerular filtration rate and renal blood flow fall. Although the urine flow is usually reduced, uremia may develop with salt depletion even when urine volume is normal. There is evidence to suggest that this is the result both of reduction in glomerular filtration rate and of increase in tubular reabsorption or back diffusion of urea. These patients are usually anoretic or nauseated and complain of great listlessness and fatigue. Painful muscle cramps are not uncommon. As sodium depletion progresses, they may become confused, somnolent and comatose.

Although the diseased kidney is particularly vulnerable to the effects of sodium deficits, hyponatremia can produce uremia even in the absence of underlying renal disease. Uremia produced entirely by salt depletion and dehydration is rarely as severe as that resulting, at least in part, from organic renal disease. However, NPN's of 80-100 mgm. per cent are not rare, even in the absence of renal disease. Urinalysis is usually of little help in differentiating the uremia of salt depletion and dehydration from that of primary renal disease. A low specific gravity, albuminuria, and formed elements in the urine may be found in both conditions. The contribution of hyponatremia to renal insufficiency in a particular patient can therefore frequently only be assessed by correcting the hyponatremia.

In order to restore to normal the concentration of serum sodium, sodium must be administered in an amount necessary to restore osmolar concentration, not only in the extracellular space, but throughout total body water. The reason for this is that any rise in the extracellular concentration of

sodium causes water to shift out of cells to re-establish osmotic equilibrium. This transcellular shift of water dilutes the sodium present in and confined to the extracellular space. If our patient has a serum sodium of 120 mEq/L and we want to give enough to restore it to 135 mEq/L, we would need (135 — 120) × (Body water) = (15) × (60% of Body weight). In a 70-kilo patient, this would equal (15) × (60% × 70 kilos) = (15) × (42) = 630 mEq. This should be given in a minimum volume of water, as a 3% or 5% solution. If glucose in water is administered, or if the patient is permitted to drink water, the effect of the hypertonic saline will obviously be nullified. For this reason, fluids should be restricted for a day or so after the hypertonic infusion is given. In any case, it is best to give reparative fluids in divided doses rather than all at once.

The response to appropriate treatment is frequently striking. The patient becomes more responsive and alert. His blood pressure rises and urinary flow increases. The NPN begins to decline and may continue to fall for several days. Unfortunately, not all patients can be expected to improve. Some patients with extensive pulmonary disease have chronic hyponatremia.[24] In these patients, hyponatremia causes no symptoms; its correction is therefore without apparent benefit. In patients with severe intractable congestive heart failure who are massively edematous and hyponatremic, the prognosis is usually poor. Although some do respond satisfactorily to the injection of hypertonic saline, the majority do not. They merely dilute their body fluids again and increase their edema when they are finally permitted to drink water.

ALKALOSIS

Chronic alkalosis, as seen in patients with peptic ulcer who ingest enormous amounts of sodium bicarbonate, may be associated with severe reversible disturbances in renal function.[4] The mechanisms involved are not completely understood. When alkalosis is induced and maintained over a period of days or weeks, there is a fall in glomerular filtration rate and renal blood flow, an increase in the NPN, a decrease in PSP excretion, and impairment of the ability of the kidneys to concentrate the urine. These functions slowly return to normal after the alkalosis is corrected. It is interesting, incidentally, that a comparable degree of acidosis does not have a similarly deleterious effect on renal function.[18]

POTASSIUM DEPLETION

The effects of alkalosis may in part be due to a concomitant depletion of the body stores of potassium. Loss of body potassium is known to be harmful to the kidneys. Rats kept on a low-potassium diet develop microscopic

lesions in their kidneys and evidence of nitrogen retention.[10] Patients with chronic potassium depletion resulting from diarrhea may show renal and cardiac lesions post-mortem, and during life may have a fixed urinary specific gravity which returns to normal when their potassium stores are repleted.[22]

Patients with pyloric stenosis, who vomit large quantities of acid stomach contents or habitually ingest enormous amounts of reabsorbable alkali, demonstrate strikingly how alkalosis and potassium loss can occur simultaneously, act synergistically to impair renal function, and act to perpetuate each other. Alkalosis per se tends to promote a greatly increased renal excretion of potassium.[2] In association with a decrease in food intake and food absorption resulting from vomiting, cellular stores of potassium can be seriously depleted. This in turn promotes extracellular alkalosis in two ways: by inducing a transfer of H^+ ions from extracellular fluids into cells,[5] and by impairing the ability of the kidneys to excrete an alkaline urine.[3] These patients may be admitted dehydrated, hyponatremic, alkalotic, with low serum chloride and high bicarbonate, and with an elevated NPN. Despite systemic alkalosis and dehydration, their urine is usually acid and of low and fixed specific gravity. It may contain albumin and casts. Correction of hyponatremia and of dehydration with saline usually results in some improvement, but alkalosis and elevation of the non-protein nitrogen frequently persist until potassium salts are added to the repair solutions. Even then, although alkalosis rapidly disappears, renal function as measured by blood NPN, excretion of PSP, and concentrating ability, improves only slowly over a period of weeks and sometimes months. Patients in this condition may have uremia fully as severe as that seen in the terminal stages of chronic glomerulonephritis. NPN's as high as 225 mg. per cent have been reported, with complete recovery by the end of a year.

HYPERCALCEMIA

Hypercalcemia, with increased excretion of calcium in the urine, can damage the kidneys in three ways: (i) by stone formation, leading to obstruction and infection in the lower urinary tract; (ii) by deposition of calcium in the collecting tubules, in distal tubular cells, and in the interstitial tissues of the kidney, producing the x-ray picture of diffuse nephrocalcinosis; (iii) finally, hypercalcemia may be associated with impaired renal function even in the absence of calcium deposition demonstrable by x-ray.

Patients with peptic ulcer who have ingested large amounts of milk, calcium oxide and calcium carbonate for years occasionally develop a syndrome consisting of an elevated serum calcium with normal serum phos-

phorus and alkaline phosphatase, and metastatic calcification in the soft tissues, the kidney, and the cornea and conjunctiva.[9] If a low-calcium diet is prescribed, there is slow regression of metastatic calcium deposits and improvement in renal function. A reliable physical sign of this condition is ocular calcification seen with a slit lamp in the cornea and conjunctiva. The use of the new chelating agents,[25] which bind calcium and are excreted rapidly in the urine, may become important in the treatment of metastatic calcification, but their value is limited in the presence of renal insufficiency because they are excreted much more slowly by damaged kidneys.

Two other disorders characterized by hypercalcemia which may produce reversible renal insufficiency are *Vitamin D intoxication* and *hyperparathyroidism*. Vitamin D intoxication has been seen most commonly as a by-product of the treatment of rheumatoid arthritis with high doses of Vitamin D. Fifty thousand units or over per day for as little as three months has been reported to produce metastatic calcification and renal impairment which improves to a variable extent when Vitamin D is withdrawn.

The loss of renal function associated with hyperparathyroidism may be very extensive. Deposits of calcium in the kidneys may produce permanent scarring and irreversible renal damage which may progress even though the hyperparathyroidism is cured. When these advanced changes have not occurred, the improvement in kidney function following parathyroid resection may be dramatic.

ANEMIA

It is known experimentally that severe anemia not only places a load on the circulatory system by increasing cardiac output, but also decreases renal blood flow and glomerular filtration rate and promotes the formation of edema.[8] In some patients with chronic nephritis and uremia the rapid and recurrent development of anemia is the chief presenting problem. When the hemoglobin falls below 7-8 gms. per cent, they experience palpitations, become dyspneic and enfeebled, and may develop edema or angina while renal function tends to deteriorate further. The anemia is usually normocytic and normochromic. It has been shown that it is due not only to depression of the formation of red cells by erythroid elements of the bone marrow, but in some patients to an increased rate of destruction by hemolysis.[13] The anemia of renal disease does not usually respond to ferrous sulfate and must be treated by periodic transfusions—not to a normal 14 gms. of hemoglobin, but to the point where anemia no longer produces symptoms. These patients have an increased tendency to develop transfusion reactions, which may further damage their kidneys. To minimize reactions, trans-

fusions should be of washed red cells and given slowly, with the tubing warmed. The administration of cobalt chloride has been reported to produce reticulocytosis and a rise in the red count and hemoglobin in some patients.[31] Unfortunately, effective doses of cobalt produce nausea and vomiting in all but a few.

SELF-LIMITED CAUSES OF RENAL INSUFFICIENCY

In addition to those reversible causes of renal deterioration which can be actively removed or improved by the physician, there are certain self-limited causes of renal insufficiency in which the damage will resolve and a remission take place, if only the patient does not die in the meantime. In the experience of every older clinician who has interested himself in such cases there are recollections of patients at death's door, with papilledema, uremic frost and uremic pericarditis, in coma, acidotic, with Küssmaul respirations, who recovered, left the hospital, and lived several years, sometimes apparently without direct relation to any therapeutic effort on the part of the doctor. For want of better understanding, some of these rare occurrences may be thought of as *acute exacerbations in the course of chronic nephritis.* The most common causes of acute, self-limited reversible renal insufficiency are *acute glomerulonephritis and acute tubular necrosis* (lower nephron nephrosis).

Acute tubular necrosis. The pathology of fatal acute tubular necrosis has been described by Dr. Jean Oliver and associates.[17] As they point out, tubular necrosis and tubular disruption are not confined to the lower nephron, but occur in spotty distribution throughout the kidney in all portions of the proximal as well as the distal tubules. In addition to toxic necrosis of proximal tubular cells, in which the basement membrane is spared, permitting regeneration, there is ischemic destruction and disruption of the entire tubular wall, including the basement membrane, creating actual holes in the renal tubule through which tubular urine can leak back into the interstitial fluid of the kidney.

Acute tubular necrosis[28] may be caused by the *ingestion of poisons* such as mercuric chloride, diethylene glycol, or carbon tetrachloride. More commonly in hospital practice it is seen *following a period of shock* in which there has been extensive tissue destruction or hemolysis. Transfusion reactions are perhaps the most common preventable cause of acute renal insufficiency with oliguria in a general hospital. The typical pattern is one of profound reduction in urinary flow within a few hours of the precipitating event. The onset of oliguria may be delayed for three or four days after exposure to toxic chemicals like CC14. Although occasionally oliguria may

be absent, or be so transient as to escape notice, it usually progresses until a minimum flow, sometimes less than 50 ml. per day, is reached on the second or third day. During the remainder of the oliguric phase, urinary flow increases slightly but progressively from day to day until it exceeds 350-400 ml./day. Thereafter it increases more rapidly, and typically, though not universally, there is a profuse diuresis. Despite the striking oliguria, complete anuria over the course of two or three days is a rarity in this disorder and it should at least direct attention to other possibilities—obstruction of both ureters, bilateral renal arterial occlusion, and acute glomerulonephritis. The oliguric phase is usually over after 10 days to two weeks. Cases have been reported with recovery after as long as 23 days of oliguria. If urine volume remains below 300 cc. for longer than three weeks, it is likely to be associated with other types of renal disease.

The chemical changes in the blood during the oliguric phase of acute renal insufficiency vary considerably according to the state of the patient prior to the acute insult, the amount of tissue destruction at the time of injury, and the therapy the patient has received. If the patient has been chronically ill or malnourished prior to having, say, a transfusion reaction which precipitates oliguria, the NPN is likely to rise rather slowly, and serum potassium may rise only slowly or not at all. An entirely different picture is seen in previously healthy people—for example, in wounded soldiers, who may undergo severe crushing injuries complicated by infection, prolonged periods of shock, and transfusion with huge quantities of outdated blood. Here protein breakdown is tremendously accelerated, nitrogenous wastes accumulate rapidly in the blood, and by the fifth to seventh day the danger of potassium intoxication may be a very real one.

The therapeutic objective in the treatment of this form of acute renal insufficiency is to maintain the patient in as good condition as possible until the tubules can heal and diuresis can occur. The following simple principles are important:[27]

I. *Replace losses.* A dilemma is frequently presented by a patient who has had no urine for several hours following a procedure which *may have caused* acute tubular necrosis (e.g., following postpartum hemorrhage) or by a patient admitted to the hospital with no urine and no reliable history. The question is whether or not to start immediately restricting fluids to the daily insensible loss. The assumption is too frequently made that there is in fact a state of acute tubular necrosis, when the reduced flow of urine may result only from inadequate replacement of fluid and electrolyte losses. Replacement of lost blood, rehydration of a contracted extracellular fluid, correction of hyponatremia, and the provision of enough water to make

urine may cause a gratifying increase in the urine flow and eliminate the whole question of renal shutdown. If the urine flow does not increase, nothing is lost. If the serum sodium is not low, and even if the patient does not look obviously dehydrated, it may be advisable to infuse 1,000 cc. of 10% glucose over an hour or so as a therapeutic test.

II. *Restrict fluids* to insensible loss plus urinary and gastro-intestinal losses. In an oliguric adult, without vomiting or diarrhea, this usually equals about 750 to 1,000 cc. per day. A great danger during the oliguric period is that of overhydration, with resultant peripheral and pulmonary edema. If fluids are restricted, this danger can be minimized. At the same time, underhydration, which has its own deleterious effects upon the circulation, should not be permitted to develop. All fluids which enter the patient must be counted. We recently saw a case in which fluids were restricted carefully to a liter of 10% glucose a day, except for a slow drip of a liter of saline a day containing nor-epinephrine. That was "for the blood pressure," but it caused edema to develop nevertheless.

III. *Minimize protein breakdown* with an intake of at least 100 grams of glucose per day. This can be done by infusing 750-1,000 cc. of 10-15% glucose, slowly and in at least two portions, morning and afternoon. Whether or not greatly increased caloric intake in the form of fat is worthwhile in terms of protein-sparing action is still a subject for debate; however, since most of these patients sooner or later develop nausea and vomiting, a really high caloric intake is frequently impossible.

IV. *Treat potassium intoxication.* Exactly when rising levels of potassium in the serum become dangerous in terms of causing cardiac arrest is somewhat unpredictable. It varies with serum concentrations of other electrolytes, with the rapidity of the rise in serum potassium, and with other and as yet unknown factors. It should be emphasized again that not all patients with oliguria develop rising potassium levels; in the experience of this department they have been notable by their absence. However, when a serum potassium of 7 mEq/L or above, associated with high peaked T waves in the electrocardiogram, occurs early in the oliguric phase, the indication par excellence exists for dialysis on an artificial kidney or for peritoneal dialysis. A high serum potassium may be depressed temporarily by infusions of glucose, and its deleterious effects on the heart ameliorated temporarily by injections of calcium salts or by infusions of hypertonic saline. Potassium can be removed from the body by use of cation exchange resins in the ammonium cycle, administered by mouth or rectum. This is an uncomfortable, laborious procedure, which is rather inefficient as far as

removal of potassium is concerned, and is not applicable to patients with abdominal wounds or operations.

Once diuresis is established, attention to the patient, far from relaxing, should redouble. Urinary losses of water and electrolytes must be replaced or hyponatremia and even hypopotassemia may develop. The NPN usually continues to rise for several days. Hypertension may progress, cardiac failure become prominent, pulmonary edema may occur, and convulsions are not uncommon during the first few days of diuresis. Pyelonephritis, frequently initiated by an indwelling catheter, often becomes a problem at this time. Anemia is usually present and muscle wasting and weakness are prominent.

If the patient survives the period of diuresis, renal function gradually returns. Anemia gradually disappears; this does not seem to be accelerated by administration of ferrous sulfate. In some patients the most careful studies several months after discharge show no abnormality of renal function. In others, disturbances in ability of the tubules to concentrate urine and to secrete PAH are apparent as long as a year after the acute injury.

Acute glomerulonephritis. The measures used to tide a patient over an oliguric episode in acute glomerulonephritis do not differ from those employed in acute tubular necrosis. Residual streptococcal infection of the throat or elsewhere should be searched for and treated. Meticulous mouth care is very important, since these patients are prone to develop infections of the mouth and neck tissues and parotitis. If vomiting occurs, the patient should be fed parenterally until there has been no vomiting for at least 24 hours. Even cracked ice given to a patient with nephritis who is vomiting may only perpetuate the vomiting, deplete him of sodium, and cause hyponatremia. Finally, since congestive failure is so frequent in acute glomerulonephritis, the use of salt restriction and digitalization in the presence of edema should be emphasized.

GENERAL THERAPEUTIC MEASURES IN RENAL DISEASE

Rest. The value of bed rest as an adjunct in the treatment of reversible uremia has a physiological rationale in the increased renal blood flow and rate of glomerular filtration which are known to occur while lying down as opposed to sitting or standing up and walking about.[28] This may be important in the appropriate case by supplying just that increment of renal function which may be crucial in reversing renal decompensation.

Diet. The question of whether a diet low or high in protein should be prescribed in chronic nephritis has been a controversy for years. It has been suggested that a low-protein diet may permit resolution of reversible renal

damage and retard further progression of renal decompensation.[1] Actually, appetite is so poor in most of these patients and nausea such a frequent complication that it is usually hard to get them to take a diet containing normal amounts of protein. From a practical standpoint, therefore, the question is whether to prescribe a diet containing ¼ to ½ gram of protein per kilo per day, or a diet of 1 to 1½ grams per kilo per day. Experimental evidence bearing on the subject is conflicting.[19] It is possible to accelerate renal damage in partially nephrectomized rats by a diet very high in protein; however, the proportion of protein in these diets is far above what is feasible to make a patient eat in a comparable clinical situation. Extremely *low* protein diets also cause renal necrosis in growing rats. The level of blood urea will undoubtedly be higher on a high protein diet than on a diet low in protein, but a high blood urea per se does not cause symptoms and is only important as an easily measured guide to the competence of the kidneys. Finally, it is obvious that chronic nephritis is a wasting disease and it seems unwise to restrict protein and further starve a patient whose serum and tissue proteins are already depleted. The reasonable attitude would seem to be to permit patients to eat a normal diet as high in protein as they have appetite for.

CASE ILLUSTRATIONS

The following case summaries illustrate two unusual causes of reversible renal insufficiency. In the first patient, the correct diagnosis was not appreciated and the patient died.

A 60-year-old man was admitted to the hospital in advanced uremia and acidosis, with Kussmaul respirations and white uremic frost on his lip. His blood pressure was normal; he was so emphysematous that his heart sounds could not be heard. The temperature and white count were never elevated. Urine volume was 4-500 cc. per day. Albuminuria was present and there were red cells in the urinary sediment. Despite rehydration, digitalization, and treatment of a urinary infection with streptomycin, he died after two weeks. At autopsy he had subacute bacterial endocarditis of his mitral valve and severe associated nephritis.

The next case represents a gratifying example of a rare form of renal insufficiency which has proved reversible.

A 40-year-old man was admitted with abdominal pain, nausea, and vomiting. His blood pressure was 156/100. The urine showed albumin, red cells, and granular casts. After overnight dehydration, its maximum specific gravity was 1.010. PSP excretion was 6% in two hours. The NPN was 53 mgm. per cent on admission but rose in one week to 122 mgm. per cent, during which time the patient became progressively more disoriented. At this time his serum potassium was found to be at the dangerously high level of 7 mEq./L. Serum calcium was found to be 16.7 mgm. per cent, with a serum phosphorus of 3.7 mgm. per cent. A diagnosis of hyperparathyroidism was made.

With forcing of fluids the blood NPN and serum potassium declined somewhat, but x-ray and electrocardiographic evidence of pericarditis with effusion appeared. His face became edematous and his lethargy, disorientation, and confusion did not improve.

He was operated on and a large parathyroid adenoma removed. Following operation there was a striking improvement in his mental state. Serum calcium and phosphorus returned to normal. His NPN fell to normal in one week and his excretion of phenol red increased to 25% in two hours. There has been a progressive increase in his ability to concentrate his urine, and six months after operation this function had returned to normal. Albuminuria has disappeared. He is working and feels well.

SUMMARY

The prognosis in uremia is not necessarily hopeless. Treatable causes of renal impairment include congestive heart failure, dehydration, infection, obstruction, salt depletion, alkalosis, loss of potassium, and hypercalcemia. In addition, there are self-limited reversible causes of renal failure which, given time, may heal by themselves. Because of the nature of renal function, the rescue of a few nephrons, the salvage of a relatively small amount of renal function, may make the difference between progressive deterioration, with rapidly advancing uremia, and renal compensation, with a gain of months or years of comfortable and useful life for a grateful patient.

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BOOK REVIEWS

RESERPINE (SERPASIL) AND OTHER ALKALOIDS OF "RAUWOLFIA SERPENTIA": CHEMISTRY, PHARMACOLOGY, AND CLINICAL APPLICATIONS. R. W. Miner, Ed. Annals N. Y. Acad. Sciences, 1954, vol. 59, art. 1. 140 pp. $3.00.

This volume consists of twelve papers on the chemistry, experimental and clinical pharmacology, and the clinical applications of the Rauwolfia alkaloids presented at a conference on Reserpine (Serpasil), sponsored by the Section of Biology of The New York Academy of Sciences on February 5, 1954. These alkaloids, which apparently have been used for centuries by Indian physicians, are now stimulating a great deal of interest and research in the Western world with at least two objectives: application in the treatment of hypertension and possible usefulness in certain neuropsychiatric disorders.

The chemical structure of one active principle, called Reserpine, has been elegantly elucidated by chemists of the Ciba Company and is reminiscent of a well-known alkaloid, yohimbine, which elicits bizarre effects from the central nervous system. This paper is one of the most impressive in the volume.

In the hands of the experimental pharmacologist the compound seems to exert a variety of autonomic effects, referable to decreased predominance of the sympathetic branch. It is suggested in two of the papers that the site of action is probably the hypothalamus where autonomic functions are integrated. However, no direct evidence for this hypothesis is presented, except, perhaps, in the paper by Travis Winsor, who was able to block inspiratory and pain reflexes after large doses of the drug.

The clinical work presented here is provocative but, like all early clinical reports, needs to be evaluated in the light of wider experience. If one recognizes that the therapy of essential hypertension still remains empirical, then one may accept the concensus presented here that Reserpine may become the agent of choice in the chemotherapy of less severe essential hypertension, especially in combination with other hypotensive agents like hydralazine, veratrum alkaloids, hexamethonium, etc. One clinical point emerges as fairly definite, and that is that Reserpine presents no serious side effects in contrast to most other currently available hypotensive agents.

The contention in the paper by R. W. Wilkins that the slow onset of action of Reserpine is due to a part played in intermediary processes of the central nervous system seems somewhat premature, in view of the fact that no knowledge of the metabolic fate and distribution of the drug is yet available.

In the studies of the effect of Reserpine on various neuropsychiatric patients, a significant tranquilizing effect and alterations of psychic states were noted. These results are, indeed, intriguing, and, as N. S. Kline has pointed out, are indicative of the "need for long-term, large-scale evaluation of this medication."

NICHOLAS J. GIARMAN

TISSUE CULTURE. By E. N. Willmer. London, Methuen & Co., Ltd.; New York, John Wiley & Sons, Inc., 1954. xx, 175 pp. $2.25.

Dr. Willmer's second edition of this little book fulfills his stated purpose ". . . to correlate some of the numerous fragments of scattered data which have so far been obtained and to orientate them in such a way as to illustrate the more fundamental contributions which the method has made to physiological knowledge, and to show the manner in which a change of outlook has already been brought about, or in which such a change is likely to be necessary in the future."

The monograph makes no pretense of being either a cookbook or a compendium of all facets of tissue culture, although the basic principles are included and are well documented by an extensive and discriminate bibliography. Rather does it emphasize certain advantages when the method is used as a tool in the investigation of certain biological problems instead of as an end in itself. Dr. Willmer has understandably emphasized the dynamic character of cells and the dependence of their functional and morphological attributes on their immediate environment. In so doing, he has accented the need for proper biological tests to determine the nature of cells in culture rather than the reliance on form alone.

The book is well printed, its line drawings are few and simple but illustrative, and the photographs are adequate. The inclusion of a glossary of biological and chemical terms in a book designed for biologists seems unnecessary but it may prove of value to some readers.

If one wishes to learn how to do tissue culture, this book is inadequate; it is excellent if one desires to know some of the things that can be done using the method.

WILHELM S. ALBRINK

THOMAS YOUNG, NATURAL PHILOSOPHER. By Alex Wood and F. Oldham. New York, Cambridge University Press, 1954. 350 pp. Illus. $6.00.

Thomas Young's genius made him eminent in every field of scientific endeavor. The man had as many facets as a diamond, and indeed, was the centerpiece in the British scientific crown, though unappreciated in this light by many of his contemporaries. A list of his investigations and accomplishments would fill an encyclopedia, as in fact they did, particularly the *Encyclopedia Britannica,* to which he was a contributor.

The transition from natural philosophy to physics took place when natural philosophers forsook the comforts of the drawing room and set up their lathes. Thomas Young was one such philosopher. He had the happy faculty of being drawn into controversial scientific issues, analyzing them mathematically and building instruments to prove his findings. He made powerful friends and powerful enemies. Because of his clear insight into the problems he encountered and his modern method of presenting his facts, he was maligned, misunderstood, and deliberately misquoted. Only as a physician and as a result of his love for "the more exact sciences" did he play a

lesser rôle. This is denied by his biographer, Dr. Peacock, who ascribes Young's failure ". . . to his being too good for his profession, and to his being above certain ignoble arts, which were, as he believed, made use of by his competitors." Munk, however, says that Young ". . . did not shine at the bedside or in the practical work of his profession, and was but little followed by pupils in the wards. His colleagues and contemporaries failed to discover that success and excellence in his treatment of disease, which his biographer, Dr. Peacock would claim for him." Pettigrew says ". . . that Young was not a popular physician. He lacked that confidence or assurance which is so necessary to the successful exercise of his profession. He was perhaps too deeply informed, did not hastily form a judgment; and his great love of and adherence to truth made him often hesitate where others felt no difficulty whatever in the expression of their opinion. But in information upon subjects of his profession it would be difficult to find his equal." Young has been hailed as the father of physiological optics, and developed, at great discomfort to himself, the physics of the human eye. An outstanding contribution of Young in the field of physiological optics is the Young-Hemholtz theory of color vision.

As professor at the Royal Institution Thomas Young's work covered everything from tides to suggestions for estimating the dimensions of molecular structure. It was during this time that work on the diffraction of water waves led him later to predict the nature of light waves. The wave theory of light was probably the greatest achievement of early nineteenth century theoretical science. John Herschel, a reluctant convert to the wave theory, said that despite Newton's and Hooke's contributions to this subject, they ". . . ought not to be mentioned with the elegant, simple and comprehensive theory of Young."

The versatility of Young can perhaps be best illustrated by his interpretation and correct identification of certain Egyptian hieroglyphics, as well as the greater portion of the demotic text of the Rosetta Stone. Champollion, a rival claimant in the interpretation of the demotic script, never fully acknowledged Young's prior discovery of its phonetic nature rather than ideographic. In the long controversy which followed, Egyptologists generally favored Young, but a natural sympathy was felt for the French, from whom the British acquired the Rosetta Stone by treaty, and Young was relegated to second honors. In the light of today's understanding of the subject this is obviously an injustice.

Between the years 1817 and 1825 Young contributed articles to the *Encyclopedia,* writing on those subjects of interest to him, which were many and varied. He wrote biographies in which, even in the shorter ones, an admirable summary of the life is given, and a careful and detailed bibliography. This latter not only enumerates the published papers of each author but summarizes their contents. Young's two most outstanding biographies are those of the French mathematician Lagrange and of the Cambridge classical scholar Porson.

Young's interests were such as to permit him to work on matters of life assurance, evolving tables and formulas of life expectancy; work on standards of length and measure; work for the Admiralty on methods of ship-

building which the Admiralty acknowledged: "Though science is much respected by their Lordships and your paper is much esteemed, it is too learned." Young was also appointed as Superintendent of the Nautical Almanac and Secretary of the Board of Longitude. In this he was unable to satisfy the demands both of the navigators and of the astronomers, incurring the displeasure of both.

A bibliography of the writings of Thomas Young shows seven works, seventy-four papers published. Undoubtedly there were written also hundreds of letters on as many subjects to the great men of his day.

JOSEPH J. CERNY

HISTOPATHOLOGIC TECHNIC AND PRACTICAL HISTOCHEMISTRY. 2d ed. By R. D. Lillie. Bethesda, Md., The Blakiston Co., Inc., 1954. 501 pp. $7.50.

For its new 2d edition this text has been revised and enlarged to give greater coverage on newer histological and histochemical procedures, at the same time retaining time-tested techniques. These methods are presented in an understandable manner, and include important details usually omitted in histological procedures. Most of the techniques presented have been worked out in Dr. Lillie's Laboratory and are given with suitable variations. At the end of each procedure, the results to be expected are listed, a very practical idea. References are also given, enabling one to refer to the original author.

A glance at the table of contents, especially the first four, will show that a great deal of thought was given to clarifying the usual stumbling blocks encountered in the preparation of histological sections.

This text goes a long way in presenting histological methods in a scientific manner and not as "cookbook" recipes. It will be a welcome addition to the histological laboratory.

F. A. PUTT

WORLD MEDICAL PERIODICALS. L. T. Morton, Compiler. Prepared under the auspices of UNESCO and WHO. Geneva, WHO and UNESCO, 1953. xvi, 237 pp. 12s6d.

World Medical Periodicals, an alphabetical list of 3,908 entries compiled by Mr. L. T. Morton of the *British Medical Journal,* proves an excellent guide to current periodicals in the medical and biological sciences and is a step forward towards the standardization of bibliographical citation in medical literature.

Titles in this publication include all current medical journals throughout the world which have been traced, all medico-biological periodicals regularly surveyed by certain abstracting agencies, and well-known journals which ceased publication during the period 1900-1950. The list includes

those periodicals devoted to pharmacy, odontology, and veterinary medicine. The entry for each title gives its name, place of publication, language or languages of publication (if not apparent from the title), frequency of publication, symbols indicating which of the principal abstracting services regularly survey the journal, and the *World List* abbreviation of the title. Irregular publication is indicated by (x), since many journals are constantly changing not only in title but also in frequency. The abbreviation of the title is based upon the code of rules introduced by the *World List of Scientific Periodicals,* as modified by the International Organization for Standardization.

The list is particularly valuable for its excellent indexes, one by subject and the other by country. Under broad subjects the serial numbers of the corresponding periodicals are entered. It is relatively simple to determine the number, exact titles, and places of publication under any given subject. Both the preliminary matter and the indexes are trilingual, entries being given in English, French, and Spanish.

The symbols used for abstracting agencies are simple and adequate, as well as the few necessary abbreviations denoting the numbers for former or more current titles of periodicals. An asterisk (*) placed before a title indicates that a journal has ceased publication or has been temporarily suspended.

The list is easy to use with its simple alphabetical arrangement, and the existing plan of regular issues of revised editions increases its value as an important tool to all concerned with medical documentation and publications. It is compact and readable, with the pages arranged in three columns: entry, number, and title and abbreviation. There are 212 pages of text and 25 pages of indexes.

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